DETECTION AND GENOTYPING OF HEPATITIS C VIRUS AMONG DIALYSIS PATIENTS IN TIRUNELVELI MEDICAL COLLEGE

DISSERTATION SUBMITTED TO

In partial fulfillment of the requirement for the degree of

DOCTOR OF MEDICINE IN MICROBIOLOGY

(Branch IV) M. D. (MICROBIOLOGY)

of

THE TAMIL NADU DR. M. G. R MEDICAL UNIVERSITY

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TIRUNELVELI- 11

APRIL 2017

BONAFIDE CERTIFICATE

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DECLARATION

I, **Dr.R.POORNAKALA** declare that, I carried out this work on **"DETECTION AND GENOTYPING OF HEPATITIS C VIRUS AMONG DIALYSIS PATIENTS IN TIRUNELVELI MEDICAL COLLEGE**" at the Department of Microbiology, Tirunelveli Medical College, I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, or diploma to any other University, Board, either in India or abroad.

This is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D Degree examination in Microbiology.

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PRINCIPAL INVESTIGATOR: DR.R.POORNAKALA, MBBS.,

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Dear , Dr.R.Poornakala, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 10.12.15.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9.	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
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1. The approval is valid for a period of 2 year/s or duration of project whichever is later
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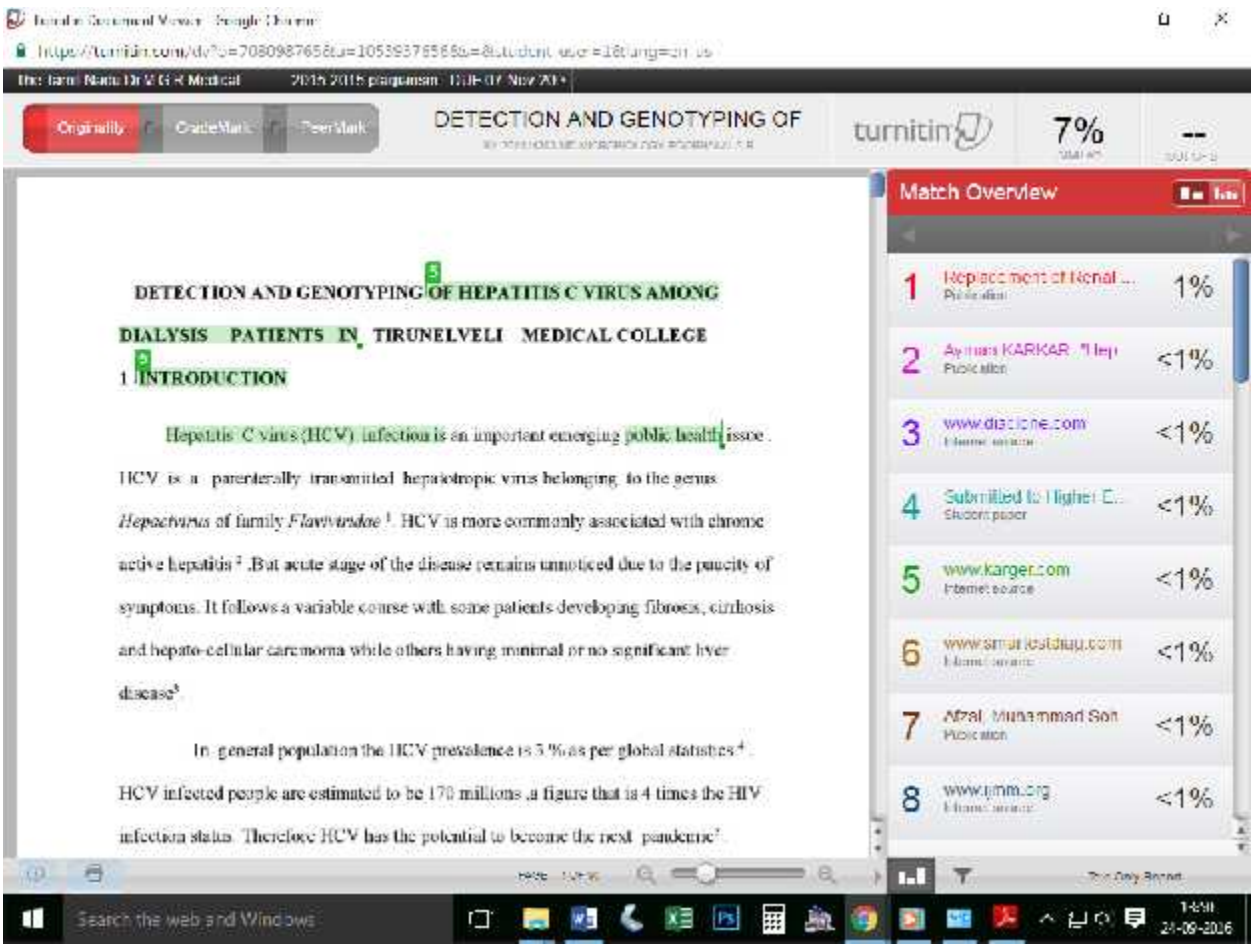

DETECTION AND GENOTYPING OF HEPATITIS C VIRUS AMONG DIALYSIS PATIENTS IN TIRUNELVELI MEDICAL COLLEGE

1 INTRODUCTION

Hepatitis C virus (HCV) infection is an important emerging public health issue. HCV is a parenterally transmitted hepatotropic virus belonging to the genus *Hepacivirus* of family *Flaviviridae* [1]. HCV is more commonly associated with chronic active hepatitis [2]. But acute stage of the disease remains unnoticed due to the paucity of symptoms. It follows a variable course with some patients developing fibrosis, cirrhosis and hepato-cellular carcinoma while others having minimal or no significant liver disease[3]

In general population the HCV prevalence is 3 % as per global statistics [4]. HCV infected people are estimated to be 170 millions ,a figure that is 4 times the HIV infection status. Therefore HCV has the potential to become the next pandemic[5].

ACKNOWLEDGEMENT

I am grateful to The **Dean, Dr. K. SITHY ATHIYA MUNAVARAH,M.D.,** Tirunelveli Medical College and, Tirunelveli Medical College hospital Tirunelveli for permitting me to carry out this study.

I extend my sincere thanks to **Prof.Dr.C.Revathy M.D.,** for her valuable guidance and support during the study period.

I would like to express my sincere thanks to **Prof. Dr. S. Poongodi @ Lakshmi M.D.,** for her constant guidance and encouragement given to me throughout this study.

I am highly indebted to **Prof. Dr.Ramesh Babu M.D.,** whose sincere guidance and encouragement were a source of strength for the successful completion of this study.

I extend my sincere thanks to **Prof.Dr.Sorna Jayanthi M.D.,** for her great guidance and encouragement given to me throughout this study.

I would like to express my sincere thanks to all my Assistant Professors **Dr.B.Cinthuja M.D., Dr. G.Velvizhi M.D., Dr. G.Sucila Thangam, M.D., Dr V.P Amudha M.D., Dr.I.M Regitha M.D., Dr.S.Gowri M.D., Dr.M.Kanagapriya M.D.** for their valuable suggestions, guidance and support throughout the study.

I would like to thank all my colleagues, **Dr.P.Anbumathi, Dr.V.Indumathi, Dr.S.Prarthana, Dr.R.Shiny, Dr.A.Umamaheswari,**

Dr.Suyambu Meenakshi, Dr.Punitharanjitham, Dr.Jeya Ganguli, Dr.Ambuja Sekar, Dr.Gracia paul, Dr.Sai Shruthi Iyer, Dr.Umamaheswari , Dr.Maya kumar, and Dr. Manimala for their support and cooperation rendered during the work.

I extend my thanks to all staff members, Department of Microbiology for giving full cooperation and timely help in carrying out the laboratory studies.

Special thanks are due to my Messer **Mr.V.Parthasarathy, Mr.V.Chandran, Mr.S.Pannerselvam, Ms.S.Santhi, Ms.S.Venkateshwari, Ms.S.Arifal Beevi, Mr.A.S.Abul Kalam, Ms.A.Kavitha, Ms.T.Jeya, Ms.K.Sindhu, Mr.Murugesan, Mr.K.Umayavel, Ms.Sreelakshmi** and are other supporting staffs for their services rendered.

I am greatly indebted to **Mr. Heber** for his expert guidance in statistics. Finally I thank the Almighty for without Him nothing would have been possible. I extend my thanks to all the patients for their participation and kind co-operation throughout the study period.

Finally, I extend my whole hearted thanks to my beloved parents, friends, family members, my husband **Dr.L.Senthilrajaperumal** my son **P.S.Karthicklaxman** for their silent sacrifices, support and words of encouragement, which were a source of inspiration for the successful completion of this study.

ABBREVIATIONS

CKD	-	Chronic Kidney Disease
HCV	-	Hepatitis C Virus
ELISA	-	Enzyme linked immunosorbent assay
ICT	-	Imunochromatography Test
RIBA	-	Recombinant ImmunoBlot Assay
IFN	-	Interferon
ESRD	-	End Stage Renal Disease
UTR	-	Untranslated Region
NS	-	Non- Structural Protein
E1,E 2	-	Envelope glycoproteins
RT-PCR	-	Real Time Polymerase Chain Reaction
HCVcAg	-	HCV core Antigen
HCC	-	Hepatocellular Carcinoma
ALT	-	Alaninie aminotransferase
WHO	-	World Health Organisation
EHM	-	Extrahepatic manifestations
IDU	-	Intravenous Drug Users
CHC	-	Chronic Hepatitis C
ER	-	Endoplasmic Reticulum

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1 .INTRODUCTION

Hepatitis C virus (HCV) infection is an important emerging public health issue . HCV is a parenterally transmitted hepatotropic virus belonging to the genus *Hepacivirus* of family *Flaviviridae* [1]. HCV is more commonly associated with chronic active hepatitis [2] .But acute stage of the disease remains unnoticed due to the paucity of symptoms. It follows a variable course with some patients developing fibrosis, cirrhosis and hepato-cellular carcinoma while others having minimal or no significant liver disease[3].

In general population the HCV prevalence is 3 % as per global statistics [4] . HCV infected people are estimated to be 170 millions ,a figure that is 4 times the HIV infection status. Therefore HCV has the potential to become the next pandemic[5] . Annually, around 3- 4million people are newly infected[6]. HCV infection causes cirrhosis and hepatocellular carcinoma .These complications are fatal in about one million cases globally [7]. There are about 12.5 million people infected with HCV in India [8]. Main sources of HCV infection include injection drug abuse, chronic dialysis ,organ transplantation, blood product transfusion, occupational exposure, unprotected sexual contact and vertical transmission[9].

In dialysis patients , HCV infection is common .This is one of the most important cause of liver disease in patients on renal replacement therapy . Chronic kidney disease(CKD) is found in approximately 10 % of general population . CKD is defined by reduction in glomerular filtration rate for three months or more and / or

proteinuria[10]. It is associated with increased cardiovascular mortality,decreased life quality ,cognitive dysfunction and rised health care costs. Dialysis is commonly used as renal replacement therapy for end stage renal disease (ESRD) patients. About 2.1 million patients are estimated to be in need of dialysis and is expected to increase annually by 7 % worldwide[11].

Dialysis patients have a considerably increased HCV prevalence compared to general population. This is due to prolonged vascular access ,frequent hospitalization and blood transfusion ,contaminated dialysis equipment and their co-morbid condition. In patients on dialysis the HCV prevalence varies between 1% to 85 % globally[12] and 3% -45% in India [13]. Chronic HCV infected individuals on dialysis have a 25 % increased mortality rate in comparison with their HCV negative counter-part[14]. Compared to uninfected dialysis patient, a HCV infected dialysis patient is 1.4 times more likely to die.[15]

Dialysis duration, frequency of blood transfusions and interpersonal horizontal route of transmission are contributing factors for HCV infection in dialysis patients[13]. Higher prevalence is seen in haemo-dialysis (HD) patients compared to peritoneal dialysis (PD) patients. The reasons include domiciliary location of therapy ,no vascular access and lesser requirement of blood transfusions in peritoneal dialysis.

HCV is a small,enveloped,single stranded positive sense ribonucleic acid virus (RNA) virus . It was discovered in 1989 and was the first virus to be detected by

molecular techniques. The lack of a vigorous T-lymphocyte response and mutagenic potential of the virus lead to chronicity of infection. More than 6 major genotypes and 100 subtypes of HCV have been identified so far. Genotype 1 is the most commonly distributed type throughout the world .More prevalent genotype isolated from Northern,Eastern,Western India is genotype 3 .In Southern India genotype 1 is mainly distributed .Genotypes 2,3 show better response to anti-HCV treatment.Genotype 1 and 4 show poorer response to therapy. The extensive heterogenecity in HCV explains the lack of response to therapy and prevents effective vaccine development[16].

The diagnosis of HCV infection is based mainly on two categories of laboratory tests. They include serologic assays to detect HCV antibodies and tests to quantify HCV RNA. Both assays are done to minimize the false- positive and false-negative results.The most sensitive tool to detect HCV infection is antibody detection test. But window phase is longer in these dialysis patients as they are severely immuno- compromised .So in early phase of HCV infection,this antibody detection test may be negative . HCV-RNA detection by Polymerase Chain Reaction (PCR) is immensely accepted as a gold standard procedure for the diagnosis of current HCV infection . Designing the therapeutic strategies depends on both genotyping and viral load assessment . Genotyping of HCV is a strong predictor of response to anti-HCV treatment[17] . So, there is a need not only to find out HCV prevalence but also to find out the genotypes of the virus prevalent in dialysis patients.

Hence , the present study was conducted in this centre during the study period of 8 months detected the prevalence and genotypes of HCV . This study will also help to formulate therapeutic strategies . Real time PCR(RT-PCR) are being done, this will give an idea about prevalence of false negative anti –HCV by Enzyme linked immunosorbent assay (ELISA) in the presence of HCV infection which is indicated by PCR .This study will also evaluate the influence of various parameters like age, sex, dialytic duration,type adopted , number of blood transfusion, serum transaminases levels in relation to HCV infection in dialysis patients.Thus, this study helps to add knowledge to evaluate the methods to reduce HCV transmission in chronic dialysis patients in future.

2. AIM AND OBJECTIVES

- To find out the prevalence of hepatitis C virus infection in dialysis patients.
- To evaluate the rapid immunochromatographic(ICT) assay with Enzyme linked immunosorbent assay(ELISA) in anti – HCV antibody detection.
- To detect HCV –RNA by quantitative PCR and evaluate ELISA in comparison to PCR.
- To assess the distribution of genotypes for therapeutic and epidemiological purpose.
- To analyse the association of various attributes in HCV transmission.

3. REVIEW OF LITERATURE

3.1 HISTORY

Hepatitis C virus discovered in 1989 was the highly elusive causative agent of post transfusion hepatitis. After 6 years of intensive investigation for the viral etiology of parenterally mediated non-A, non-B hepatitis (NANBH) using molecular biological methods, a single c DNA clone was isolated from experimentally infected chimpanzees. Michael Bradley and Daniel Bradley at Centre for disease control (CDC) identified the Hepatitis C virus as a relatively lipid coated virus.[16]

3.2 PROPERTIES OF THE VIRUS

3.2.1 STRUCTURE

HCV is a small, spherical, enveloped virus with a single stranded RNA genome of positive coding polarity of about 30-60 nm in diameter .Viral particle surface contains virally encoded lipid bilayered envelope with glycoproteins E1,E2 arranged as spikes of 6nm. HCV has an icosahedra shaped nucleocapsid covering the core RNA .[18]

3.2.2 HCV GENOME ORGANIZATION

The HCV RNA genome comprises of 9397 nucleotide bases in length, of which more than 98 % contains protein coding sequence. This genome has a single open reading frame and are translated to yield a large polyprotein of 3011 amino

acids. The poly protein is cleaved into 3 structural and 7 non structural proteins by proteases after post translational and co-translational modifications.

Table 1 : Proteins in HCV genome and their functions

PROTEINS	FUNCTIONS
Core protein	Nucleo capsid formation, RNA binding
E1,E2	In virus attachment
P7	Ion channel
NS2	Cysteine proteolytic enzyme cleaving NS2 and NS3 junction
NS3	Proteolytic and helicase activity;Target for antiviral agents
NS4	Co factor for NS3 protease
NS 5A	RNA binding ,replication complex formation ,combating innate cell defenses
NS 5B	RNA dependent RNA polymerase, HCV genome copying while replication.

It also has highly conserved non coding regions at both 5’ and 3’ ends .In the 5’ untranslated region (UTR) end the genome uncapped and internal ribosomal entry site is essential for translation. The structural(S) and non-structural proteins(NS) are located at the 5’ and 3’untranslated regions respectively. The structural components of the virus particle are core C, Envelope glycoproteins E1(gp 35) and E2(gp 70) which are required for infectious viral particles formation.E1 and E2 are major antigenic sites and their varying nature is essential in immune pathogenesis and chronicity of infection.

3.3 HCV REPLICATION

The primary sites of HCV replication are hepatocytes. The extra-hepatic sites are bone marrow stem cells and B-lymphocytes. Entry mechanism for HCV into hepatocytes is complex . Replication mainly in the cytoplasm of infected cell. The LDL receptor, heparin sulphate, CD 81,human scavenger receptor, tight junction proteins including claudin and occludin proteins are involved in the initial attachment of HCV into the cell.

HCV is a lipo-particle and serves as a lipoprotein which is responsible for evasion of immune containment and clearance. After attachment, penetration and uptake into a cellular endosome coated with clathrin, acidification alters the dimension of envelope proteins that ends in endosomal membrane formation .The viral RNA is released into the cytoplasm,acts as messenger RNA which helps in cap - independent polyprotein translation .Translation results in cooperation with rough

endoplasmic reticulum (ER) by internal ribosome entry site and the polyprotein undergoes further co-translational proteolytic cleavages in a series manner.

The core-proteins present inside the cyto plasm:E1 and E2 are secreted into ER lumen and stay attached to the membrane.A replicase complex of NS3-NS5 forms membranous web associated with lipid droplets derived from ER.This complex recognizes 3' end and produce negative –strand copy of the genome . Replicase identifies the other end of genome following that duplex RNA helps in further production of numerous copies of genomic RNA with positive polarity by acting as a template .Packaging genomic RNA as new viral particles, later extruded into ER leads to virus release via vesicular secretory pathway.

Half-life of HCV virion in the bloodstream is 45 min. Around 10^{12} virions are generated per 24 hours in an infected humans .Rate of HCV production is 10-100 fold higher than HIV[19] leading to escape mutants generation causing chronicity of infection. HCV is a heterogenous virus with genetic diversity[20].Chimpanzees are the only species other than man that can be infected with HCV and thus have proved important in natural history of HCV.[21]

3.4 HCV GENOTYPES

There are six major genotypes with more than 100 subtypes among HCV. The genotypes differ from each other by 15-25% in their RNA nucleotide sequence. HCV Genotype vary in their geographical distribution. Genotype 1 is the most common genotype present globally, being predominantly found in America, Japan, Korea,

Australia & New Zealand. Genotype 3 is highly prevalent in India. Genotype 2 is mostly found in Mediterranean and Far East countries. Genotype 4 is predominantly found in the Middle-East and Egypt. Genotype 5 is commonly found in South Africa while genotype 6 is found in Hongkong, Vietnam ,Australia.[22] . . Type 2 Diabetes millietus and disease severity is strongly associated with type 1,which has efficient replication ability . Genotype 3 is associated with hepatic fatty changes.[23]

3.5 CONCEPT OF QUASI -SPECIES

Within any given patient these subtypes of HCV circulate as complex closely related viral population known as Quasispecies. The genetic diversity of the HCV virus could influence the spontaneous cure rate after acute infection by evasion of the host immune response . Neutralising HCV antibodies are short lived and no immunity appear after acute HCV infection.[24]

3.6 CLINICAL FEATURES

Hepatitis C virus causes both acute and chronic infection.

3.6 .1Acute HCV infection

Manifested mainly as asymptomatic The mean incubation period is about 2-12 weeks . About 20 – 30% patients presented with malaise , fatigue , right upper quadrant pain , nausea , dark urine and jaundice. Spontaneous resolution occurs in 40% individuals .Spontaneous disappearance of HCV RNA in dialysis patents is reported as 1%[25]

HCV RNA is found in blood within 2 weeks of exposure with elevation of serum alanine transaminase and aspartate transaminase . About three fourth persons harbor HCV and progress to chronic infection.Anti-HCV antibodies develop within 8-10 weeks of exposure and present forever .[25]

3.8.2 CHRONIC HCV INFECTION(CHC)

In people with chronic HCV infection, disease progression is variable; it leads to slow but progressive deterioration of liver function in as many as 70% cases. Fibrosis in CHC occurs as an effect of hepatic stellate cells activation by cytokines. 20% will develop cirrhosis and its related complications such as portal hypertension and about 1% to4 % of cirrhotic cases may develop hepatocellular carcinoma (HCC) every year, but after 20 to 30 years of infection [26]

Serum alanine transaminase (ALT) levels eventually fluctuate irrespective of symptoms with fairly constant levels of serum HCV RNA. CHC accounts for 40 % cases of chronic liver disease [27].

Faster progression is associated with risk factors like ingestion of alcohol ,older age, Immunodeficiency ,longer duration of infection, more complex quasi species diversity ,advanced histologic stage and grade, concominant other liver diseases ,increased hepatic iron, male sex ,obesity ,HIV and HBV co-infection. The 10 year survival rate is about 80 % , mortality occurs at a rate of 2-6% per year in a compensated cirrhotic HCV infected patient and decompensation occurs at a rate of 4-5 % per year[28].

3.6.3 HEPATOCELLULAR CARCINOMA

Primary HCC is typically a late complication of CHC.Clinical features represent sudden worsening of cirrhotic features with right upper quadrant pain.Serum α-fetoprotein levels are elevated.Ultrasonogram and CT scan reveal an intrahepatic mass and liver biopsy is needed.

3.6.4 EXTRA-HEPATIC MANIFESTATIONS

HCV is responsible for extra-hepatic manifestations (EHM)by three mechanisms-[29]

1.HCV is able to replicate cytopathically in extra-hepatic cells

2.Trigger an auto immune mechanism against antigens expressed on non hepatic cells. 3.Fomation of immune complexes causing vascular deposition.

40 % -74 % of HCV patients develop atleast one EHM during their course of diaease[30] . Chronic HCV infection have been associated strongly with essential mixed cryoglobulinemia, membranoproliferative glomerulonephritis, porphyria cutanea tarda[31].Other diseases include kerato-conjunctivitis sicca, moorens corneal ulcer, lichen planus, autoimmune thyroiditis, idiopathic pulmonary fibrosis ,lichen planus, necrolytic acral erythema, cardiomyopathy, HCV related thrombocytopenia and small joint polyarthritis.[32]

3.7 PREVALENCE OF HCV IN DIALYSIS PATIENT:

3.7.1 PREVALANCE -GLOBAL SCENARIO

The prevalence of HCV infection in CKD patients on dialysis is higher than that in the general population and it can be atleast 5 times higher than general

population.[33].It varies widely from country to country ranging from 5% to 60 %[34] .The incidence of HCV, in new patients starting renal replacementtherapy, ranged from 3% to 7%, and reported seroconversion rates while on treatment in between 1% and 16% per year.[35]

Dialysis Outcomes and Practice Patterns Study (DOPPS)survey revealed the mean prevalence of 13.5 % in dialysis patients in seven developed countries[36]. A study by Vidales –Braz et al in 2015from Southern Brazil revealed the prevalence of HCV was 18.24%[37].Khan et al in Pakistan(2011) demonstrated the prevalence of HCV around 29 %[6] . Hinrichsen et al (2002) demonstrated the prevalence of HCV around 7%[38] .

Selcuk et al in Turkey (2005) studied and demonstrated the prevalence of anti-HCV in HD was 26% while in Continuous ambulatory peritoneal dialysis (CAPD) was 16 %. And in the same study detected HCV –RNA in HD and CAPD patients was 49% and 42% respectively.[39] Estimated dialysis related risk is 2% per year for HCV infection.[11]

3.7.2 PREVALANCE -INDIAN SCENARIO:

The prevalence of HCV among renal replacement patient is around 3% - 45% in India[13].

Jasuja et al in 2009 studied the prevalence of HCV as 27.7 %[40].A study from Prakash et al in 2014 demonstrated the prevalence of HCV was 57.14%..[41] A study from Punjab by Neerja Jindal and colleagues demonstrated HCV infection among HD was 56.25%[42]

Vikas makkar et al in 2014 revealed the prevalence of anti-HCV was 18.54% [43].A study by Chawla et al in 2005 estimated the HCV prevalence as 3.61 %.The prevalence of HCV among renal transplant recipients was 28.9 %-42 % from various studies in India. [13] .The rate of sero –conversion in HD patients was 1.38 %-1.9 %/year.[2]

A study by Das et al revealed 3.61 % of HCV infection in HD,4.83 % in PD and 6.1 % among renal transplant patients.[44]

3.7.3 PREVALANCE IN SOUTH INDIA :

A study by Hegde in 2014 studied the seroprevalence of HCV in HD was 10.6% [45] .A study by Asima banu revealed lower seroprevalence of HCV about 0.02%[46] .Chigrupathy et al demonsrated the incidence of anti-HCV as 23.5 %.[47] . A study by Reddy et al in 2006 demonstrated the prevalence of HCV infection was 13.5% [48] .A study from Bangalore by Shalini Ashok Naik et al reported the prevalence of HCV about 15 % [49] .A report by Agarwal stated varying prevalence rate ranging from 4.3%-13.5% from different metropolitan cities among dialysis patients from our country[50] .

3.7.4 PREVALANCE IN TAMILNADU : .

Limited number of studies were only available in Tamilnadu regarding dialysis patients on a comparative analysis between serology and RT-PCR. A study by Murthy et al[51] revealed 7.5 % of HCV prevalence in dialysis and renal transplant patients. Kanagapriya et al [52] reported the sero prevalence of HCV was 5.9% out of 121

hemodialysis patients in 2011.A study from Coimbatore by Kumar et al reported the prevalence of HCV as 12.4% [53].

3.8 INCUBATION PERIOD

Incubation period is about 15-160 days with mean of 50 days.HCV infection is of insidious onset.

3.9 ROUTES OF TRANSMISSION

The modes of transmission are percutaneous exposure to infected products (Transfusion of blood and its components ,injection drug use(IDU),sharing of needles and equipments) Non apparent percutaneous routes (piercing of ear,body, tattooing ,circumcision ,surgical /dental procedures using improperly sterilized instruments) ,nosocomial (accidental needle prick injury while surgery, treatment ,interpersonal spread during dialysis) ,preventive health campaigns (immunization ,microtransfusions for low birth weight new borns) sexual ,vertical transmission [54]

3.9.1 PARENTERAL ROUTE OF TRANSMISSION

Common mode of transmission for spread of HCV infection is through contaminated blood and blood products. Mandatory screening was introduced in India from 2002.After that HCV prevalence was dropped to major extent .A study by Jaiswal reported higher prevalence of HCV of 21% in multi transfusion individuals[55]. A study from Kolkata reported the prevalence of HCV was 13% by Neogi et al in 1997 among donors [56]. Rehan et al stated about 6.9% of patients had HCV [57] . In a study from Delhi by Agarwal et al [50] revealed the presence of HCV was 26.6% in multi-transfused children

Intravenous drug users(IDU) accounts for 55 % - 65 % of all infections[1] .Estimated rate of HCV infection in intravenous drug users is around 20 % per year[18].Saha et al in 2000 was given higher prevalence of HCV 92% in IDU groups from Manipur[58] . A study from Chennai by Mehta et al reported the prevalence of HCV about 55%among intravenous drug abusers[59].

Unsafe therapeutic injections, contaminated injection equipment ,reusable syringes by unlicensed practitioners of medicine were considered as major risk factor in several developing countries .Seroprevalence of HCV among them to treat kala-azar was 31.1%[60]. Rates of HCV positivity in cadaveric donors vary from 1% -11.8 % in different countries [61] .

3.9.2 NOSOCOMIAL TRANSMISSION

Previous admissions in hospital is an main parameter in HCV infected patients. Spread is more common here due to sharing of contaminated equipments in between patients and inadequate disinfection procedures.Around 2 % to 20 % higher prevalence in hospitalized patients [62] .When vigorous practicing of universal precautions applied in dialysis units ,no seroconversions were found even when the patients used same equipments in a dialysis units. Stringent blood testing and isolation of dialysis machines helped in mitigation of HCV transmission.New incidence is related to prevalance. Jasuja et al reported that dialysis units with less than 19% prevalence had an incidence of 2.5% annually. Also when prevalence is more than 60 %,units had 35.3 % incidence[40].

3.9.3 NON –APPARENT PARENTERAL TRANSMISSION

3.9.3.1 TATTOOING

A study in Taiwan reported 12.6% among 87 young healthy tattooed men had HCV antibody positivity [63.] In a study by Khaja et al, exposure to tattooing had raised HCV transmission of 2.8% [64.]

3.9.3.2 ACUPUNCTURE

Acupuncture can cause HCV infection by inexperienced persons. More prevalent in Korean chronic liver disease patients. Also there is a significant association with this is demonstrated by Shepard et al in Taiwan in 2005[65].

3.9.3.3 HEALTH –CARE WORKERS

HCV infection from infected patients to health care individuals was well reported and analysis of nucleic acid amplification has confirmed this mode of spread. Mean risk of infection may be in the range of 0.3% - 3 % after needle prick .Dentists were having significantly higher prevalence of HCV of about 5.4% from Rajasthan[62] .

3.9.4 NON-PARENTERAL TRANSMISSION

3.9.4.1 VERTICAL TRANSMISSION

The vertical transmission rate is 4%-7% per pregnancy globally .Generally transmission occurs at birth presumably through blood contact..The HCV prevalence among pregnant women is 0.1 %-2.4 % [62] . A study by Gowri et al reported 0% prevalence in ante natal group [66] . Breast feeding carries no risk of spread. The disease in new borns is mild and free of symptoms.

3.9.4.2 SEXUAL AND HOUSE HOLD TRANSMISSION

Direct evidence from studies stated limited with definite risk of HCV spread sexually. Its occurence has been associated with prolonged and repetitive exposure. Transmission by this route is less efficient than other viruses. A study by Bhattacharya et al [67] demonstrated the HCV prevalence of 6 %. with STDs in South India .Inter-spousal transmission rate is very low (0.23%) per annually .Among siblings and house hold contacts has prevalence of 4 % in patients with chronic liver disease.

HCV was also transmitted in non-injecting drug users [68].

3.10 Risk factors of HCV infection in CKD patients:

HCV infection is common in patients undergoing regular dialysis .Dialysis and renal transplantation are the modalities of treatment in CKD patients. Around 20 % population undergo renal replacement therapy in developing countries. Hemodialysis is the most commonly utilized mode of renal replacement therapy and cardiovascular disease is the major reason of mortality in these patients .The atherogenic role of HCV through aggravation of metabolic syndrome and dyslipidemia contribute to cardiac disease. A study from Tehran by Zahedi et al in 2012 documented main causes of CKD as hyperglycaemia and hypertension [69]

Analysis of various factors in HCV transmission

3.10.1 Type of dialysis :

Hemodialysis (HD) has increased HCV infection rate compared to peritoneal dialysis[70] (PD). A study by Johnson et al [71] observed higher HCV prevalence in HD group in comparison with PD patients on testing large study group of 2 lacs patients.

HD unit has more HCV infection rate due to numerous vascular access,periodic blood transfusion and nosocomial transmission to which CKD patients are subjected continuously.

3.10.2 Duration of dialysis

Duration on HD |is well recognized as a predisposing parameter here[72]. Brazilian study reported HD cases of longer duration had 13.6 fold increased risk of HCV positivity[73].

Tehranian study in 2012 demonstrated the age of HCV positive patients around 42-50yrs with male preponderance. Mean duration of dialysis was above 55 months[69].

In another study by Oliveria da silva et al in 2013 from Southern Brazil demonstrated the time period of positive anti-HCV patients on dialysis was around 82 months[74]. In a Vietnamese study in 2015 , mean duration of dialysis was 80 months among positive cases [11]. In a study by Vikkas Makkar et al reported that mean duration of dialysis in anti-HCV positive patients was 67.63 weeks compared to non reactive patients in which mean duration was 43.5 weeks[43] . HD for more than six years has 11 fold increase in HCV infection in comparison to HCV patients with less than 3 yrs of dialysis[75] · Frequency of dialysis. and number of dialysis sessions are having significant association with HCV infectivity cases .

3.10.3 Blood transfusion

The number of blood transfusions infused was well correlated with increased HCV-positivity consistently[76 , 77] in these patients .One unit of blood transfusion increased the odds of HCV infection by 1.16 times in these patients.

Recently some studies could not find correlation between blood transfusion and HCV transmission [9,77].

3.10.4 Other risk factors

Other risk factors include older age, dialysis in multiple centers,. a history of organ transplantation[78] , graft survival was affected by HCV positivity. hepatitis B infection, HIV infection and diabetes mellitus[79].

3.11 OCCULT HCV INFECTION

Occurs in 2 forms:

1.HCV antibody positive patients with normal levels of liver enzymes and without serum HCV RNA and

2.HCV antibody and HCV RNA negative patients with abnormal liver function

The sites of HCV-RNA occult infection that have been identified include the liver and lymphatic cells such as peripheral blood mononuclear cells (PBMCs) and dendritic cells[80] .Studies of occult HCV infection have reported detectable HCV RNA over prolonged periods of time.In the study by Vidimlisk reported that out of 109 patients ,49 patients(45%) were positive [81].

3.12 LABORATORY DIAGNOSIS

3.12.1 DIRECT DETECTION

Electron microscopy reveals spherical, approximately 40 nm in size with smooth contour. But it is expensive and unable to detect the low levels of viral particles in serum.[1]

3.12.2 ANTIGEN DETECTION

HCV antigen - an Enzyme immunoassay method (EIA) for HCV antigen is available. It detects core protein which is a phospho-protein of 191 aminoacids in length [82] .HCV core antigen (HCVcAg)is detectable throughout viraemic pre-seroconversion period. It allows HCV infection isolation 45 days earlier compared to antibody screening tests. and an average of only two days later than quantitative HCV RNA detection in individual specimens. A study by Fabrizi et al from California in 2005 observed the sensitivity and specificity was 92.7% and 97.4%. The HCVcAg ELISA efficacy ranged from 81.9% to 95.9%[83] . In another study done byKesli in 2011 from Turkey confirmed 100 % specificity and positive predictive value of HCVcAg [84] ..In patients with low HCV viral loads HCVcAg has low sensitivity for HCV diagnosis. HCVcAg acts like a HCV replication marker and current HCV infection in anti-HCV positive patients in areas lacking virologic testing. This test is rapid and inexpensive (three times less costlier)than NAT.It is needed as a supplemental or pre confirmatory test to pre confirm anti-HCV results.It has lower risk of laboratory contamination.[85]

3.12.3 ANTIBODY DETECTION

3.12.3.1 FIRST GENERATION ELISA

Kuo et al was used to detect antibodies to c100-3 epitope of NS4 antigen and was first introduced in 1990.Esteban et al demonstrated the presence of antibody to

c100-3 antigen highly prevalent in drug abusers.This test has low sensitivity and low specificity and detects antibodies in 16 weeks.[31]

3.12.3.2SECOND GENERATION ELISA

This test was introduced in introduced in 1992 and detects antibodies to core(c22-3), NS4(c100-3) and NS3(c33).It has improved sensitivity of 92-95% and detects antibodies in 10 weeks. False negativity is more in dialysis group (2.6%-7%)[80,86].

3.12.3.3 THIRD GENERATION ELISA

This test was introduced in 1996 and incorporated recombinant NS5 ,core, NS3,NS4 antigens .It detects antibodies in 4-8 weeks. It is very sensitive of 97-99% and specificity-99% . Both sensitivity and specificity depends on the antigenic type used . EIA-3 testing had excellent accuracy, with 0.26% false negative rate[78,87], and is better .

False negatives are more in immunocompromised inviduals and dialysis populations.The immunocompromised state and effect of malnutrition-inflammation complex syndrome(MICS) of dialysis patients is mainly responsible for their deficient antibody response[88].Cell mediated immune response is also dysfunctional in dialysis patients.Since there is a window period between infection and seroconversion and lower ELISA sensitivity in these patients ,a single negative ELISA cannot rule out HCV infection in these patients.This may lead to the fact that HCV-antibody EIA testing has poor sensitivity with higher rates of false negative results among HD

patients.Some studies have repeatedly revealed HCV RNA positivity in sero-negative dialysis patients [86] .A study by Pragati Chigrupati et al in Rajamundry reported the sensitivity is 100% and specificity of 67% for 3.0 Elisa[47].

3.12.4 RECOMBINANT IMMUNOBLOT ASSAY(RIBA)

This test detects recombinant HCV antigens and identifies responses to individual proteins.Human superoxide dismutase is included as a control to detect nonspecific antibodies.Recombinant antigens of HCV are coated as bands on Nitrocellulose strips and incubated with patient's serum . Individual reactivities are with peroxidase-labelled anti-IgG and colorimetric reaction

Interpretation: Reactive :> 1 antigen, Indeterminate:1 antigen, **Negative:** no Ag detected

Used as a confirmatory test in low-risk patients and HCV RNA negative cases[1].Recent infection and cross-reacting antibodies cause indeterminate results. The main advantages are exclusion of false-positives with screening ELISA and it correlates well with HCV viremia patients. It is highly specific. Disadvantages are relative decreased sensitivity, high cost, and complex and long duration of procedure. Occasionally, RIBA intermediate or negative samples can be positive for HCV RNA – infections and RIBA can be positive or indeterminate in resolved infection.

A study from Iran by Alavian et al [89] demonstrated that in RIBA ,13.2% of patients were confirmed as HCV-positive.

In one Jordanian study by Bdour et al in 2002 observed that out of 98 anti-HCV patients determined by EIA ,94 % were also reactive in an immunoblot assay [90].

3.12.5 CHEMILUMINESCENCE ASSAY

This microparticle – based test detects HCV core antigen and antibodies more swiftly in acute infection.It has more false positives and indeterminate results. Hence it is used as a screening test alone [1].Recombinant HCV antigen coated paramagnetic micro particles and assay diluent are combined followed by addition of .After washing, anti-human antibody acridinium-labeled conjugate is added .Following another wash pre-Trigger and Trigger solution are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light unit (RLU). A Vietnamese study in 2015 by Duong et al revealed 6 % of HCVcAg positivity out of 113 HD patients [11].

3.12.6 IMMUNOCHROMATOGRAPHY TEST

It is a rapid, simple and point of care test. Results are read within 15 min. Recently , Tajeldin et al revealed the superiority of Elisa over ICT in the diagnosis of HCV infection[91]. A study from Battool et al in 2009 from Lahore[92] and Hayder et al[93] reported high rate of false positive result and less than 1 % false negative rates. A study from CDC, Atlanta in 2012 demonstrated the sensitivity and specificity of this test as 86%-99% and >99 % respectively[94] .

3.12.7 HCV TRIDOT TEST

It is a rapid, visual and sensitive qualitativ test for antibodies detection to HCV in human plasma or serum.This fourth generation test has been developed using modified HCV antigens representing the immunodominant regions of HCV antigens. It utilizes a unique combination of Recombinant antigen and Synthetic peptides for core,NS3-5 to selectivity identifies all sub types of HCV .The device include two test dots T1 and T2 and a built in quality control Dot C. It has a high degree of sensitivity and specificity.

3.12.8 MOLECULAR METHODS

This test directly detects HCV RNA and is able to distinguish current infection and past infection. These tests are needed for viraemia (HCV RNA) detection ,viral load and genotype to guide treatment decisions. Also, viraemia detection is needed for effective diagnosis of HCV infection in acute stage of hepatitis and widely used in diagnosis of infection in immunosupressed individuals who fail to mount a detectable antibody response.It is used for the confirmation of serologically positive individuals and also needed in serologically negative individuals but with unexplained liver disease. Viraemia can be detected in serum after 21 days .[95]

Nucleic acid tests are divided into qualitative tests (Reverse transcriptase polymerase chain reaction and transcription mediated amplification) and quantitative tests (branched DNA and quantitative RT-PCR). Qualitative assays include target amplification methods and quantitative assay includes signal amplification methods.

Various factors influencing HCV quantification are assay used,time to serum separation, testing laboratoty, storage temperature, collection tube.Viraemia decreases after dialysis and reverts to normal baseline after 2 days . Major reasons are heparin usage dialytic membrane nature, pressure effect and increased plasma IFN levels during the dialysis [87.]

The current CDC recommendations for HCV screening in HD patients include testing for anti-HCV and serum ALT on admission, ALT on a monthly basis and anti-HCV once in six months. Some studies reported a high rate of false-negative serologic testing with 3 possible patterns of viremia with clinical relevance: i) Transient viraemia with acute resolving hepatitis ii)intermittent viraemia iii) Persistent viraemia progressing to chronicity.

The frequency of positive viraemia with HCV antibody negative reported as 0% to 12% among HD subjects in several studies[96,97] .

3.12.8 .1 REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR)

The preferred target for PCR is 5' non-coding region which is highly conserved Region. It is considered as the gold standard in the diagnosis of HCV infection [27.] It is also suitable for HCV screening to prevent the transmission of this disease. Detection limits < 50 IU/ml .False positive results can occur due to false positivity .False negative results can occur due to intermittent viraemia. Imperfect storage and handling of samples lead to failure of HCV RNA detection in nearly 40 % of cases[98.]

NESTED PCR done using multiple primer sets within a single PCR reactions.

3.12.8 . 2 TRANSCRIPTION MEDIATED AMPLIFICATION (TMA)

Detection limits to 5-10 IU/ml. Sensitivity is more than 98% and Specificity is 99.6% . This is mainly done for screening blood donor and organ transplantation.

3.12.8.3 Quantitative Real-time PCR

Real time PCR has a detection limit of 10-15 IU/ml. To measure baseline viral load and during treatment to assess on treatment response. Mainly using Taqman assay.[99]

3.12.8.4 BRANCHED CHAIN ASSAY(bDNA assay)

Detection limit < 615U/ml .Pre treatment quantification and response to treatment can be detected.[18]

3.12.9 HCV GENOTYPING

HCV genotyping determination is useful in assessing the treatment response. Commonly NS5 region used which is highly variable. HCV strains by genotyping the samples with positive PCR by sequencing the 5'UTR with NS5B was very good primer and having better sensitivity [100] .Available methods for this tests are gene sequencing, line probe assay using reverse hybridisation, DNA enzyme immunoassay ,Real time reverse transcription PCR using type specific primers, PCR -Restriction fragment length polymorphism(RFLP).Genotype can also be determined by competitive EIA detecting specific antibodies. Direct sequencing is the most

reliable method. Type specific PCR assays require only a single amplification step and are simpler. The first type-specific genotyping assay was described by Okamoto et al. Pre –treatment assessment of genotype is essential for dosing and duration of treatment to obtain a sustained clearance of viraemia. It does not influence disease presentation or severity of disease. HCV genotypes 1, 4, 5, and 6 show poorer treatment response and require prolonged duration of treatment. Some studies identified HCV genotype 1b as the most prevalent subtype in patients HD or CAPD in Turkey by Selcuk et al [39] and by Sukanya et al[101] in Vellore respectively. In a study by Perez *et al* [102] reported findings that HCV genotype 1a was the most prevalent subtype in patients receiving HD, followed by genotype 1b .Genotyping is recognized as a major predictor of response to anti-viral therapy.

3.12.10 Liver biopsy

Severity of liver disease is recommended before initiation of treatment of HCV. Complications are oozing at puncture site, local site tenderness, Major histological changes include lymphoid follicles within the portal tracts, bile duct damage ,lobular hepatitis with lymphocytic infiltration in the sinusoids surrounding the hepatocytes ,parenchymal steatosis.

Other validated scoring systems include the Metavir scoring, Knodell score , Ishak score , Histological activity index, Scheuer score , Batts and Ludwig score and Desmet score. Immunohistochemical and electron microscopic studies revealed HCV antigens localized to the cytoplasm of infected hepatocytes.

3.12.11 OTHER INVESTIGATIONS

Serum albumin was lower, but TIBC and creatinine were higher in HCV-infected patients [103]. Presence of anti-LKM antibodies in serum represent auto immune hepatitis in chronic HCV infection.

3.12.12 OTHER LIVER MARKERS

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and gammaglutamyl transferase (GGT) are biomarkers used to monitor hepatic inflammation, though none of these enzymes are specific to the liver

Elevated levels of enzymes generally correlates with degree of viremia and increased disease activity. HCV positive patients had reduced AST and ALT levels because of hemodilution , ALT synthesis in hepatocytes is reduced , low viremia following dialysis, the generation of a hepatocyte growth factor, endogenous interferon-a, and lymphocyte activation having reduced viral action on hepatocytes[14] . Normal serum transaminases levels may be mainly because of pyridoxine deficiency, uremic toxins production, or UV-absorbing components in the blood altering the transaminase levels [104]. Liver enzymes are persistently normal in dialysis patients.

3.13 TREATMENT[105]

HCV is a treatable disease now-a-days because its genome does not integrate into the host genome. This non –integration makes it easier to eradicate HCV. Treatment decreases viral replication and may eradicate hepatitis C virus. It delays the progression to cirrhosis, and thereby decrease the frequency of hepatocellular

carcinoma, and prevent extrahepatic complications of infection. Various drugs are now available to treat HCV such as Interferon, Ribavirin and directly acting anti-viral drugs.

3.13.1 INTERFERONS(IFN)

Interferons are with antiviral, immunomodulatory, and anti-proliferative functions. Interferon- alfa induces signal transduction after optimum binding with membrane receptors thereby ends in arresting of viral replication.

The pegylated (polyethylene glycol-complexed) interferon alfa-2 and alfa-2b has reduced clearance, longer half-lives, and steadier concentrations .Interferons are excreted through kidneys and dosage must therefore be adjusted.

3.13.2 RIBAVIRIN (RBV)

Ribavirin is a guanosine analog that is phosphorylated intracellularly by host cell enzymes. It interfere with guanosine triphosphate synthesis , inhibits capping of viral messenger RNA and polymerase activity of certain viruses-HCV .

Duration of and its treatment outcome was determined by genotype .Three patterns are observed in IFN/RBV therapy.

Sustained viroloical response (SVR):	Viral RNA is not detected 6 months after end of treatment.
Non-responders	HCV RNA in high levels even after stoppage of treatment.
Relapsers	Initially respond but relapse after cessation of therapy

3.13.3 DIRECTLY ACTING ANTI-VIRALS(DAA)

3.13.3.1 HCV PROTEASE INHIBITORS

They are directly acting inhibitors of HCV replication targeted the NS3/NS4 serine protease.Boceprevir and Telaprevir are used for genotype 1 infection licensed since 2011. Simeprevir(SIM) is active against genotypes 1,2,4 and 6. Danoprevir is active against genotypes 1,4,6.

3.13.3.2 NUCLEOTIDE & NON-NUCLEOSIDE POLYMERASE INHIBITORS

The NS5B polymerase activity is inhibited by nucleoside substrates that incorporate into the growing RNA chain and terminate replication.

Sofosbuvir(SOF) is a chain terminator prodrug with broad pangenotypic activity .

3.13.3.3 NS5A TARGETING DRUGS

NS5A is a non-enzymatic phosphoprotein required for RNA replication and assembly of infectious particles. Daclatasvir is a pan-genotypic and administered once daily .

3.13.4 RECOMMENDED TREATMENT(WHO)

Genotypes 1 and 4 :48 weeks (peg-IFN,RBV)

Genotypes 2 and 3:24 weeks (peg –IFN ,RBV)

Genotypes 1,3,4: Triple therapy with PEG IFN –α, Ribavirin, SOF for 12 weeks

For IFN-intolerant pts : Sofosbuvir and Simprevir for 12 weeks

Genotype 2 : Sofosbuvir plus Ribavirin for 12 weeks

Genotype 3 : Sofosbuvir plus Ribavirin for 24 weeks.

3.14 PREVENTION

1.Using personal protective equipments(PPE) like gloves,water proof gowns .

2.Regular disinfection of the equipment circuit at the end of treatment of every patient.

3.Clean surface and surroundings maintainance.

4.Avoid instruments or drugs used in between patients.

5.Ensure proper bio-safety control programmes.

6.Training of health-care personnel.

7.Improved access to safe blood and mandatory testing of blood

8.Clean needle programmes.

9.Immunisation with hepatitis B vaccine is mandatory.

10.Peroidical monitoring of enzyme levels and other parameters are necessary in detecting chronic infection earlier.

4 . MATERIALS AND METHODS

The present study was conducted at the Microbiology department, Tirunelveli Medical College, Tirunelveli from December 2015 to August 2016 to detect the prevalence of anti –HCV antibody by rapid immunochromatographic test [ICT] , Enzyme linked immunosorbent assay[ELISA] and also to detect HCV -RNA by Real time Polymerase chain reaction [RT-PCR] among Chronic kidney disease (CKD) patients on dialysis.

4.1 Study group

A total of 100 chronic kidney disease individuals on dialysis were subjected here as study group

4.1.1 Inclusion criteria

1.Age more than 12 yrs

2. Patients with chronic kidney disease on dialysis for more than three months

4.1.2 Exclusion criteria

1. Children less than 12 years of age

2 . Acute kidney injury individuals.

4.1.3 Ethical clearance

Ethical committee clearance was obtained from the college ethical committee before the commencement of the study.

4.1.4 Consent

Informed consent was obtained from reliable informants of patients and patients who participated in the study.

4.1.5 Questionnaire

A filled up proforma regarding patients name, age, sex, area, details of any co-morbid condition, History of previous hospitalization for surgery, dental procedures, any injections, any drug abuse history, acupuncture, tattooing, history of past blood transfusions, history of anaemia, sharing of needles, razors, brushes, history of jaundice, history of spouse HCV, organ transplant were collected .Regarding dialysis, type of dialysis, haemodialysis –number, duration, frequency, nature, A-V fistula made, re-usage of dialyser, previously visited dialysis centre and all these details were collected.

4.1.6 Study sample

A sum of 100 non-repetitive serum samples were obtained from the study group. Around 4 ml of venous blood sample was collected from study cases under aseptic precautions in labelled disposable tubes. Serum was separated and collected

in serum vial by the use of micropipette and was labeled properly. Discard the hemolysed samples and collect fresh samples from those patients.

4.1.7 Storage of sample

Samples were immediately tested for HCV antibody using rapid immunochromatographic method and later stored for ELISA ,PCR and genotyping at -20 ^{o}C in a deep freezer until testing in separate aliquots.

4.2 METHODS

All 100 samples were assayed for parameters of HCV with rapid immunochromatography (ICT) , ELISA (anti-HCV) and PCR (HCV-RNA).

4.2.1 Immunochromatography card test

All the 100 samples were tested by rapid immunochromatography card test (SD BIOLINE anti-HCV antibody).

4.2.1.1 PRINCIPLE :

In general, ICT is a lateral flow assay that contains a chromatographic pad having three zones - sample pad, captureline, conjugate pad.. Colloid gold is impregnated in the conjugate pad with HCV capture antigen (core, NS3-NS5). When the specimen is placed in the sample pad, due to capillary action it flows laterally . On reaching the pad with conjugate it forms antigen-antibody complex by binding to the antibody conjugate in the pad. This complex then flows laterally to reach the capture line where

it is captured by second antibody. The presence of coloured line indicates a positive result.

4.2.1.2 Materials provided

The rapid immunochromatographic test kit has

1) Anti- HCV assay device pouched separately in a foil
2) 10 µl Capillary pipette
3) Assay diluent contains sodium azide

4.2.1.3 Anti-HCV test device:

- 1st test strip has Gold conjugates with recombinant HCV capture antigen protein gold colloid
- Test line T (as main component): Goat anti- HCV antibody
- Control line (as main component): Goat monoclonal anti-human antibody

Assay buffer included: 100 mM Phosphate buffer and Sodium azide.

4.2.1.4 STORAGE: This kit can be stored at 1-30*C

4.2.1.5 PROCEDURE

1) The test cassette was removed and placed on a clean flat surface.

2)10 µl of serum specimen was placed in sample well using a capillary pipette

3) About 4 drops (about 120 µl) of assay diluents was added in sample well

4) Results was interpreted within 20 minutes.

4.2.1.6 Interpretation of the results

1) The control line (C) was seen on the left side of result window and Colour band was seen if the kit was functioning properly.

2) Test line was seen in right side of result window.

Negative Report : Only one color band (C) in the control window was present

Positive Report : Two color bands were seen (T and C bands) .

4.2.2 THIRD GENERATION ELISA

All 100 samples were subjected for anti-HCV ELISA third generation by SD Biostandard diagnostic kit.

4.2.2.1PRINCIPLE :

Recombinant HCV antigens (core, NS, NS4 and NS5) highly specific for HCV antibody has been coated to the wells of microtitre plate provided during manufacture. Following incubation, anti-HCV in patient serum is bound to the recombinant HCV antigens. All excess unbound analyte are removed by meticulous washing following incubation. Then HRP -enzyme conjugate is added to every well. Following incubation excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue coloured complex with conjugate. The colour development is then stopped by adding acid which turns the final end product as yellow. The intensity of product coloured

complex is directly proportional to concentration of anti-HCV in samples . Reading is performed with spectrophotometer at 450 nm colorimetrically. This is an indirect sandwich ELISA for the qualitative detection at antibodies against HCV.

Table : 2 Reagents in ELISA

Material / regent	**Remarks**
Coated Microplates	96 wells with recombinant HCV antigens encoded
Enzyme Conjugate	Goat anti – human IgG with horseradish peroxidase (HRPO).
Sample Diluent	Phosphate buffer, bovine serum
Positive Control	Anti – HCV positive human serum.
Negative Control	Normal human serum.
TMB Substrate A	Sodium acetate, Hydrogen peroxide ,Gentamicin.
TMB Substrate B	Tetrametiny/benzidine (TMB)
Washingsolution (20xconcentrated)	Phosphate buffered saline
Stopping sodium	1N Suphuric acid

4.2.2.2PROCEDURE

The strip wells for negative control 3 wells, positive control 2 wells and samples were arranged initially.100 µl of sample diluent was added to all wells.10 µl of negative control 3 wells, positive control 2 wells and sample to each well was added .Shake well after appropriate sealing. Wells are incubated at 37^0 C for 30 minutes. Washing the wells 5 times with 350 µl of diluted washing solution. 100 ul

of enzyme conjugate was added to each well and cover the microplate and incubated .Again washed 5 times with same solution. Gently mix the TMB substrate A and B in equal proportion and pipette 10µl of the same to all wells .The wells were placed for 10 minutes in an incubator at room temperature. Then 100 µl of stopping solution was added to each well.

Read the absorbance of the wells with a bichromatic spectrophotometer at 450 nm, with reference wavelength at 620 nm. Absorbance was read within one hour of the assay.

4.2.2.3 Interpretation of results

Validation of assay is determined

1. If the negative control was more than 0.010 and less than 0.200

2.If the positive control was more than 1.000

4.2.2.4 Evaluation

Cut –off value was measured by adding negative control mean with 0.400.

Test reports:

1. Sample value less than cut-off value reported as anti – HCV negative
2. Sample value more than cut-off value reported as anti – HCV positive

4.2.3 Real-Time PCR

All the 100 samples tested by rapid ICT and ELISA were tested again with Real time-PCR by the kit provided by Helini Biomolecules, Chennai, India and the procedure was done as per guidelines in user manual.

4.2.3.1 Principle of Real time PCR

In Real-Time PCR the progress of amplification reaction is monitored by a camera in “real-time”. A fluorescent marker which binds to the amplified DNA is used as a marker of progress. Products produced during each cycle of the whole process is in direct proportion to the template amount prior to the run of amplification process. Every one copy of specific sequence amplified and detected in an exponential manner. As the number of gene copies increases, the fluorescence also increases which is easily detected.

The increase in the fluorescence emission in the amplification reaction may seen in real time manner by a thermocycler. Amplification plots can be constructed by computer software and can be collected during the PCR cycle.

4.2.3.2 Safety precautions

All the laboratory works were carried out as per standard laboratory procedures and Bio-safety norms in Class II Biosafety cabinet

4.2.3.3 Instruments

Vortex mixer

Refrigrated centrifuge

Thermocycler (Biorad CFX 96)

Computer for data analysis and storage

4.2.3.4RNA extraction

4.2.3.4.1 Components of RNA extraction kit

Carrier RNA (cRNA), Lysis buffer, Internal Control Template, Wash Buffer-I, Wash Buffer- II, Ethyl alcohol, Elution Buffer

4.2.3.4.2 Storage and stability

The kit can be stored at room temperature(15-25℃) for up to 12 months except carrier RNA which was stored at -20 C.

4.2.3.4.3 Principle of RNA extraction

The principle is based on optimum binding capacity of membrane based silica gel with specific coupling properties of RNA. RNase is inactivated under denaturing condition for lysis of sample initially then intact viral RNA is isolated. After addition of carrier RNA, sample RNA binds to the membrane optimally and then it binds to

the spin column after washing with wash buffers effectively. Finally, RNA of pure quality is eluted and is devoid of any contaminants.

Reagent preparation as per manufacturer guidelines

4.2.3.4.4 PROCEDURE

Extraction step:

- 1.5ml PCR tube+ add 20 µl Proteinase+add 200µl sample(after vortex)+add 200µl lysis buffer(vortex for 30 sec)
- Add 5 µl cRNA and 5µl Internal control template(ICT)
- Vortexed and centrifuged nearly 2 min at 8000 rotations and incubated (56 ·C 10 min)
- 220 µl of 100%Ethanol was added .
- Vortex followed by brief centrifugation 8000rpm for 2min
- Arrange the spin column-Transfer whole content into respective spin column.
- Vortexed for 8000rpm for 60 seconds . Same collection tube was used after discarding the flow-through .
- 500 µl of W1-Centrifuge 8000rpm for 1 min was added .Discard the flow-through and use the same collection tube.
- 500 µl of W2 was taken and centrifuged for 1 min at 8000 rpm.
- Same collection tube was used after discarding the flow-through.
- Again 500 µl of W2 was pipetted and centrifuged for 1 min at 8000rpm.

- Same collection tube was added after discarding the flow-through.
- Centrifuge empty spin column attached with collection tube-12000 rpm for 2 min
- Spin column was placed in new 1.5 ml PCR tube after discarding the collection tube.
- 60µl of elution buffer was added. At room temperature(RT) nearly 2 min incubated.
- Centrifuge 10000 rpm for 1min and the spin column was discarded

  Filtrate contains RNA. Eluted RNA was either used immediately for RT-PCR or stored at -80℃ for later analysis.

4.2.3.5 PCR amplification

4.2.3.5.1 Components of HCV- PCR kit

- Real - time PCR Master mix has essential reagents for PCR amplification like Reverse Transcriptase enzyme, dNTP, Taq DNA polymerase, Taq reaction buffer, Mgcl2, and ribolock enzyme
- HCV Primer Probe Mix - The primer probe has a forward primer and a reverse primer specific for 3' UTR region of HCV tagged with FAM as a fluorophore and BHQ1 as the quencher molecule.
- Forward Primer: 5'-GCRGAAAGCGYCTAGCCATG

  Reverse primer: 5'-ACCCAACRCTACTCGGCTA [R = A or G]

Probe: ATGGCGTTAGTATGAGTGTCGAA

- Internal Control Primer & Probe Mix to make sure that PCR inhibitors are not present in the extracted sample.
- Nuclease free water

Reaction assay:

- Change the gloves. Arrange the PCR block. Mark the wells.
- Mark as NC, QS1 ,QS2,QS3, QS4,Samples, In house quality control IHQC(HCV positive sample from previous run)
- Bring the HCV deduction mix to RT and prepare the HCV deduction mix in the 1.5ml PCR tube.

Probe RT-PCR Master mix	: 8µl
RT- Enzyme mix	: 2µl
HCV primer probe Mix	: 2.5µl
ICT primer probe Mix	: 2.5 µl

- 15µl HCV deduction mix was added in all wells.
- 10µl of nuclease free water was added to NC.
- Standards were prepared as per guidelines.
- 10µl of QS1, QS2, QS3, QS4 was added to the respective wells. Then add 10µl of samples to respective wells.
- 10 µl of IHQC was added to the last well

- Close the well with PCR block cap and remove the air bubble by tapping the well Centrifuge 8000 rpm for 2 min and load the PCR block in thermocycler.

Amplification reactions were performed on a Biorad (CFX 96-Realtime system) Thermocycler with the following thermal conditions.

Table-3 Amplification profile HCV RNA PCR

	Step	**Time**	**Temp**
	Reverse transcription	30min	42˚C
	Taq enzyme activation	15min	95˚C
45cycles	Denaturation	20sec	95˚C
	Annealing/ Data collection	20 sec	58˚C
	Extension	20 sec	72 C

Select FAM for HCV ,HEX for ICT.

Repeat selection cycle at step 3.

- Results along with amplification curves are stored in the computer and computer print outs taken for further analysis.

4.2.3.6 Result interpretation:

Ct value ≤34 with typical sigmoid shape amplification curve was taken as positive

Ct value >34 was taken as negative

4.2.4 Procedure for genotyping

Hepatitis C virus real-time PCR kit constitutes a ready to-use system for the detection of HCV genotypes usingpolymerase chain reaction (PCR). It contains reagents andenzymes for the specific amplification of 185bp region of the HCVgenome, and for the direct detection of the specific amplicon influorescence FAM channel. All genotypes are FAM labeled and external positive controls (HCV – QS1) are supplied to assist thereaction.

4.2.4.1 PRINCIPLE :

The target sequence (3'UTR) is highly conserved and shown to be a good genetic marker for HCV genotypes and can detect less than 20 copies of target template. Purified RNA was extracted as mentioned earlier.

In this study, Conversion of HCV RNA to cDNA copy by the enzyme reverse transcriptase and amplification of cDNA by HCV genotyping Real-Time PCR reaction and FAM channel amplification indicates the type of genotype present in the sample.

4.2.4.2 Components of HCV genotyping Real-time PCR kit:

1. Complementary DNA (c DNA) reaction kit

2.HCV genotyping real time PCR reaction kit contains Probe PCR master mix and type specific primer probe mix.

4.2.4.3 Universal HCV

Forward Primer: 5'-GCRGAAAGCGYCTAGCCATG

Reverse primer: 5'-ACCCAACRCTACTCGGCTA [R = A or G]

Probe: ATGGCGTTAGTATGAGTGTCGAA

4.2.4.4 Genotyping Primers:

Common Reverse Primer: ACCCAACRCTACTCGGCTA [R = A or G]

6 specific forward primer: GGCGACACTCCACCATGAT

5 specific forward primer: ATGGCGTTAGTATGAGTGTCGAA

4 specific forward primer: GAGCAACACTCCACCATGAACC

3 specific forward primer: AGGAACTWCTGTCTTCACGCG [W= A or T]

2 specific forward primer: CAAGAAAGGACCCAGTCTTYC [Y = C or T]

1 specific forward primer: CAAGAAAGGACCCGGTCGT

Bring the HCV c DNA deduction mix to RT and prepare the HCV c DNA deduction mix in the 1.5ml PCR tube

4.2.4.5 cDNA assay reaction

Components Volume: cDNA reaction mix6µl, RT-Enzyme Mix2µl, cDNA Primer M2µl,Purified RNA sample 10µl, Total reaction volume 20µl

Centrifuge PCR vials briefly before placing into thermal cycler.

Program as follows: Hold - 42°C for 60min

Use 2.5µl of cDNA for real-time PCR.

4.2.4.6 Real- time PCR reaction mix

- Probe PCR master mix of 10µl was taken in a PCR tube.
- To this ,HCV type specific primer probe mix of 12.5µl of specific types from 1,2,3,4,5 and 6 was added to each samples.
- cDNAof 2.5µl was added to all samples

Negative Control Include 2.5µl of sterile PCR grade water

Positive Control Add 2.5µl of positive control with their primer probe mix

Table-4 Amplification profile

	Step	**Time**	**Temp**
	Taq enzyme activation	15min	95˚C
	Denaturation	20 sec	95° C
45cycles	Annealing/ Data collection	20 sec	58°C
	Extension	20 sec	72° C

HCV genotype 1 / 2/ 3/ 4/ 5/ 6 = FAM channel

.FAM channel amplification indicates the genotype of the test sample.

5.RESULTS

5.1 The Study Group

A total of 100 serum samples of Chronic Kidney Disease(CKD) patients undergoing Dialysis were enrolled. This study was conducted at the Department of Microbiology, Tirunelveli Medical College Hospital, Tirunelveli done for 8 months from December 2015 to August 2016.

5.2 Statistical Analysis

All the results obtained from the study were analysed stasticially for their completeness, consistency and accuracy by the parameters like mean and percentages. Kappa value was calculated to measure the degree of agreement between three diagnostic methods. The results of rapid ICT ,ELISA and RT-PCR were compared by Mcnemar's χ^2 test and confirmed by 'Z' test of proportions. The above statistical procedures were performed by IBM SPSS Statistics 20. Significant probability value of less than 0.05 was important statistically.

5.3 Result analysis

The selected 100 study subjects were analysed based on age and sex and was depicted in Table.5

Table 5. Age and sex distribution of the study group

Age(in years)	Male		Female		Total	
	No	%	No	%	No	%
12-20	3	5	3	8	6	6
21-30	9	14	7	20	16	16
31-40	26	40	8	23	34	34
41-50	20	30	8	23	28	28
51-60	4	6	7	20	11	11
>60	3	5	2	6	5	5
TOTAL	65	100	35	100	100	100

Out of 100 patients, 65 were males. Of this, 3 (5%) were in the age group of less than 20 years,9 (14%) were in third decade and 26(40%) were in fourth decade and 20 (30%) ,4(6%) ,3(5%) were in fifth, sixth, seventh decade respectively.

Remaining 35 were females .Of this 3(8%) were in the age group of less than20 years and 7 (20%) were of third decade and 8(23%),8(23%),7(20%),2(6%) were in the fourth, fifth, sixth, seventh decade respectively.

Male to female ratio was 1.85:1.

The analysis stated that the average age of male and female was 39.4 years.

Fig.1 Age and sex wise distribution of study group

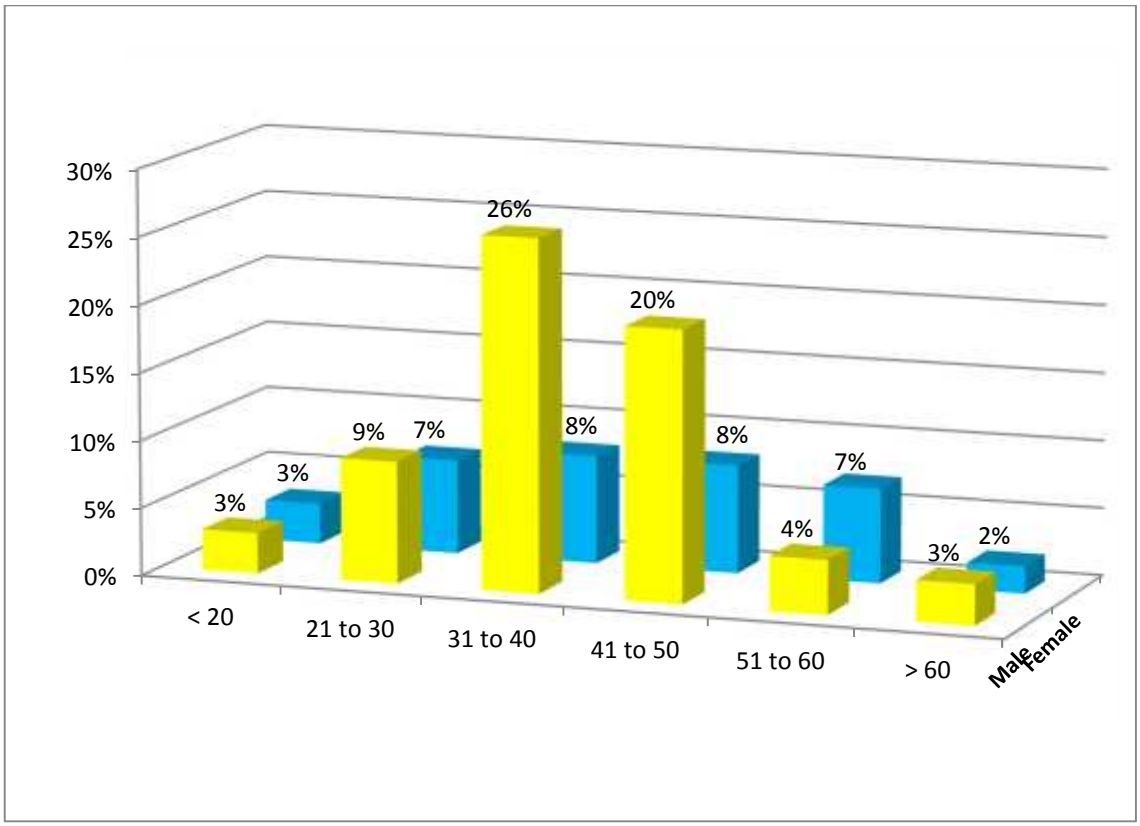


Table-6 Distribution of various causes leading to CKD in the study group:

Causes	Tested cases (n=100)	Percentage
Hypertension	51	51%
Diabetes mellietus	24	24%
Chronic glomerulonephritis	13	13%
Chronic interstitial nephritis	5	5%
Others(congenital, obstructive causes)	7	7%
TOTAL	100	100

Out of 100 cases,51 % cases were hypertensive (HTN)and 24% were diabetic(DM). These are followed by 13 % cases constituting Chronic glomerulonephritis (CGN) and then by congenital and obstructive causes and other unknown causes (7 %).Least number of cases(5%) leading to CKD were due to Chronic interstitial nephritis(CIN) as per the present study. (Table:6)

Fig.2 Causes leading to CKD among study group:

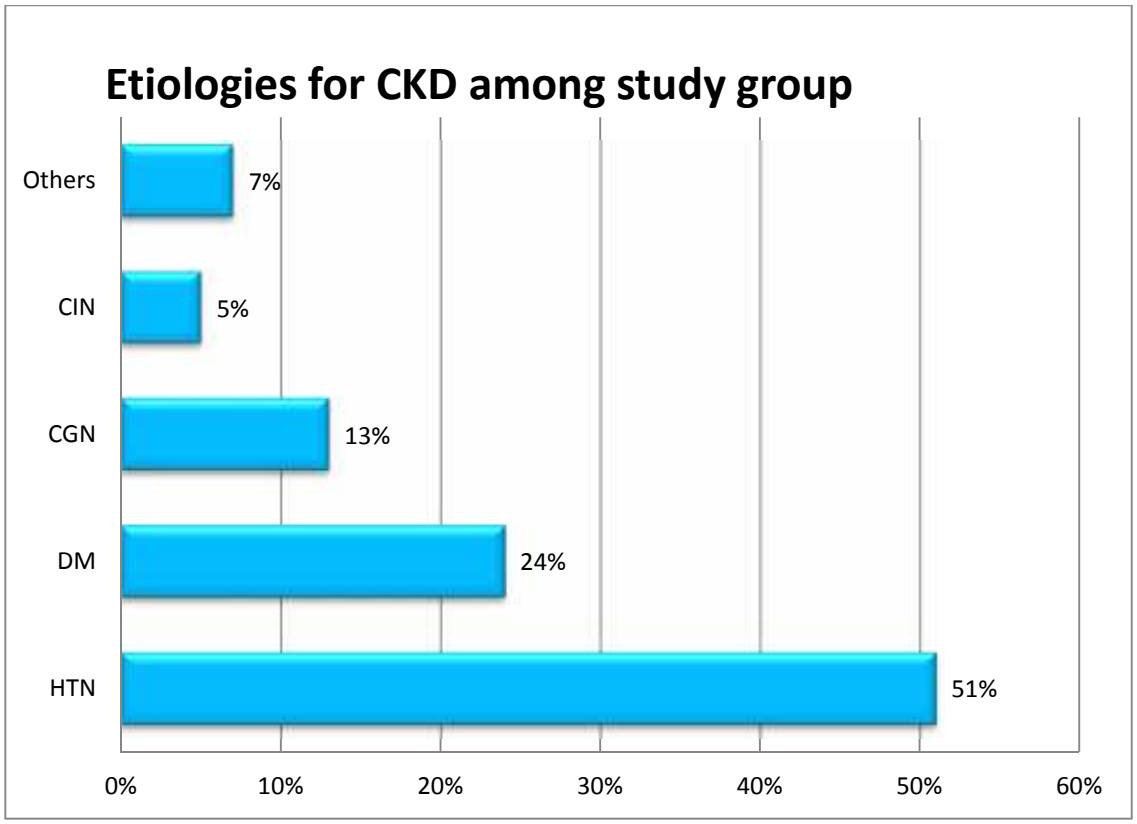


Table-7 Mode of dialysis in the study group:

Mode of dialysis	NUMBER n=100	
	No	%
Peritoneal dialysis only	13	13%
Hemodialysis only	57	57%
Peritoneal dialysis then Hemodialysis	30	30%

The table:7 and figure :3 showed the mode of dialysis in the study group. Out of 100 cases studied,57 % of cases were undergoing hemodialysis only and 30% of cases underwent peritoneal dialysis initially followed by hemodialysisand 13 % cases were on peritoneal dialysis alone.

Fig.3 Mode of dialysis among study group

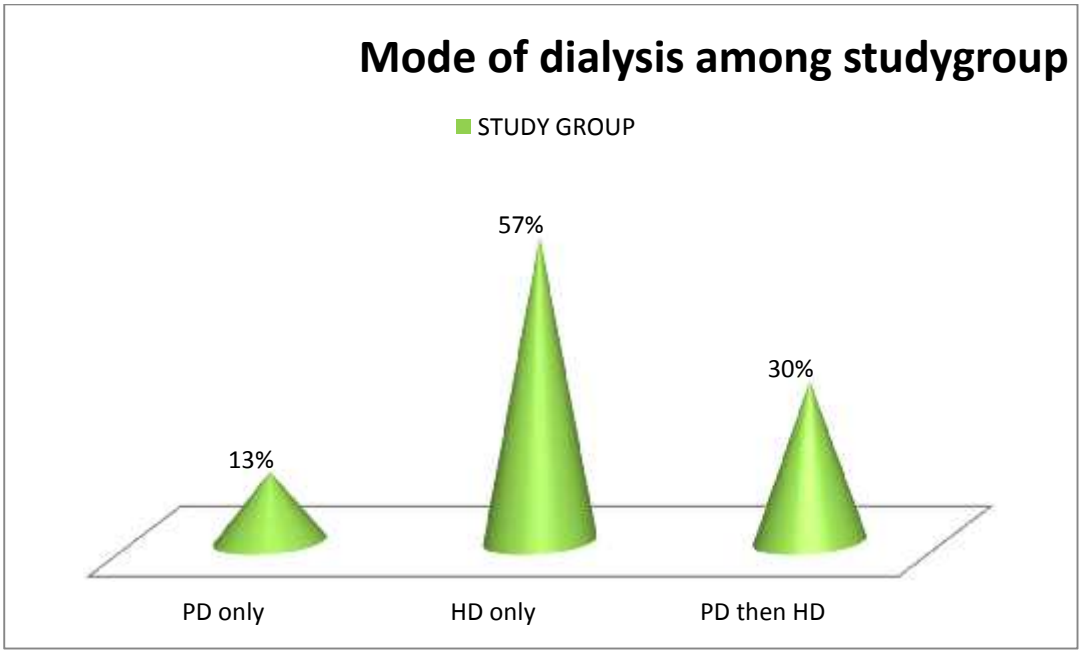


Table 8 **Detection of HCV infection by rapid ICT , ELISA and PCR.**

TESTS	Sample tested	HCV Positive		HCV Negative	
		Cases	%	Cases	%
ELISA	100	8	8	92	92
ICT		7	7	93	93
PCR		7	7	93	93

All the 100 samples were tested by three diagnostic tests-rapid ICT, ELISA and Real-time PCR for Hepatitis C infection.(anti-HCV,HCV RNA).Out of this ELISA was positive for 8% of samples while 7% were positive for ICT.Real time PCR also showed 7% positivity as shown in table 8 and figure 4.

Fig 4:Comparison of HCV detection by ICT,ELISA,Real time-PCR

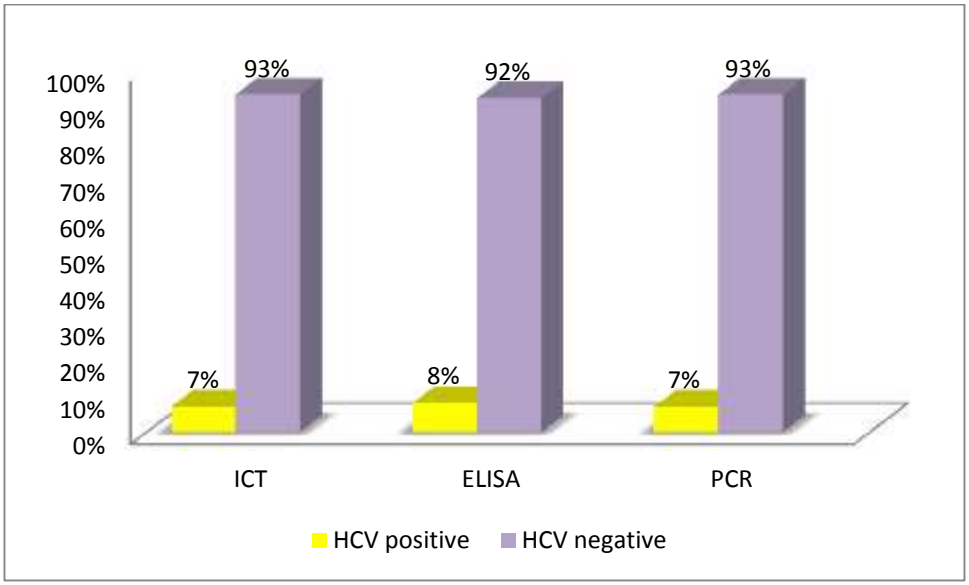


Table :9 Correlation between rapid ICT and ELISA in HCV antibody detection

ICT	ELISA		TOTAL
	Positive	Negative	
Positive	7	0	7
Negative	1	92	93
Total	8	92	100

The rapid immunochromatographic card test was evaluated for its sensitivity and specificity against ELISA ,a reference test

From the above table, sensitivity of rapid ICT was 87.5% when evaluated against ELISA, a reference test. Specificity was 100 % compared to ELISA and positive predictive value of ICT was also 100%.Negative predictive value of ICT was 98.92%

The kappa value measuring the degree of agreement between ICT and Elisa was 0.928.

Table 10 : Evaluation of ICT with PCR as reference

ICT	PCR		TOTAL
	Positive	Negative	
Positive	5	2	9
Negative	2	91	93
Total	7	93	100

The above table showed that only 5 cases were positive for both PCR and ICT . 2 cases were positive for ICT only. Another 2 cases were positive for PCR alone .91 cases were negative for both ICT and PCR. Detection of anti-HCV positive cases by rapid immunochromatographic card test was evaluated for its sensitivity and specificity against PCR,a gold standard test.

Sensitivity $=\frac{T}{T+F}$ =5/7×100 =71.4%

Specificity $=\frac{T}{T+F}$ = 91/93×100 = 97.8%

Positive predictive value $=\frac{T}{T+F}$ =5/7×100= 71.4%

Negative predictive value $=\frac{T}{T+F}$ = 91/93×100= 97.8%

The kappa value denoting the degree of agreement between ICT and PCR was 0.693.

Table 11:Evaluation of ELISA with PCR as reference

ELISA	PCR		TOTAL
	Positive	Negative	
Positive	6	2	8
Negative	1	91	92
Total	7	93	100

Detection of HCV positive cases by Elisa test was evaluated for its sensitivity and specificity against PCR as gold standard test.

Sensitivity $=\frac{T}{T+F}$ =6/7×100= 85.7%

Specificity $=\frac{T}{T+F}$ = 91/93×100 =97.5%

Positive predictive value $=\frac{T}{T+F}$ = 6/8×100 = 75%

Negative predictive value $=\frac{T}{T+F}$ = 91/92 ×100 = 98.9 %

The kappa value measuring the degree of agreement between Elisa and PCR was 0.794.

Table 12 : Consolidated results of ELISA(anti-HCV) and Real time PCR(HCV RNA)

TESTS / PARAMETERS	NUMBER	PERCENTAGE
Elisa +/RT-PCR +	6	6%
Elisa +/RT-PCR-	2	2%
Elisa - /RT-PCR+	1	1%
Total no.of infected cases	9	9%
Elisa - /RT-PCR- (Non infected cases)	91	91%

The above table showed the presence of both ELISA and RT-PCR positive in 6 % of cases. ELISA positive and RT-PCR negative (Anti-HCV positive and HCV RNA negative) were reported in 2 % of cases.

ELISA negative and RT-PCR positive was seen in 1 % of total cases. The net number of infected persons was 9(9 %). Therefore, the overall prevalence rate was 9 % in this study.

Both ELISA and RT-PCR were negative (anti-HCV and HCV RNA negative) in 91% cases of study group.

Table 13:**Age wise distribution among HCV positive cases**

Age (in years)	HCV Positive cases	
	No.	**%**
< 20	1	11%
21-30	3	33%
31-40	4	44%
41-50	1	11%
>50	0	0
Total	9	100
Mean age	30.9	

The above table:13 showed that majority of positive cases were in the fourth decade .Total of 9 positive samples tested by Elisa and PCR, 4 (44%) were in the age group of 31-40 years. This is followed by a incidence little more in third decade. The mean age among positive cases was 30.9 years.

Fig 5:Age wise distribution of HCV positive cases

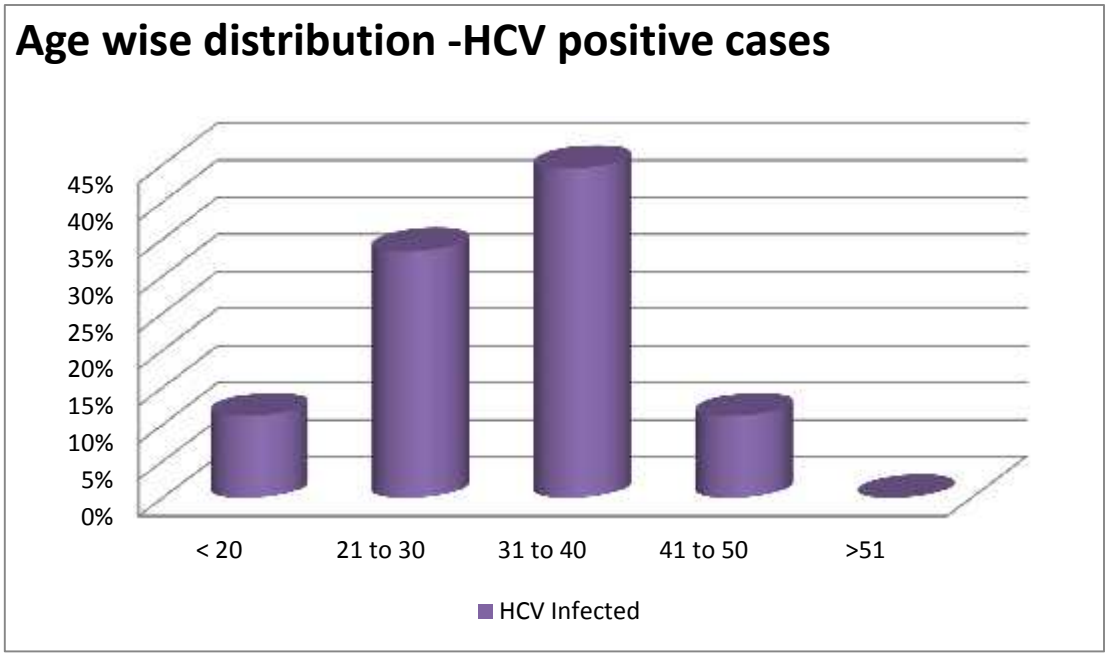


Table 14:Sex wise distribution of HCV positive cases

Sex	HCV Positive cases	
	Number	Percentage
Male	6	67
Female	3	33
Total	9	100

Sex wise distribution of HCV positive cases in the above table shows that males were more affected than females. Fig 6 showed that out of total 9 positive cases ,6(67%) were male and 3(33%) were females.

Male to female ratio among positive cases 2:1.

Fig 6: Sex wise distribution of HCV positive cases

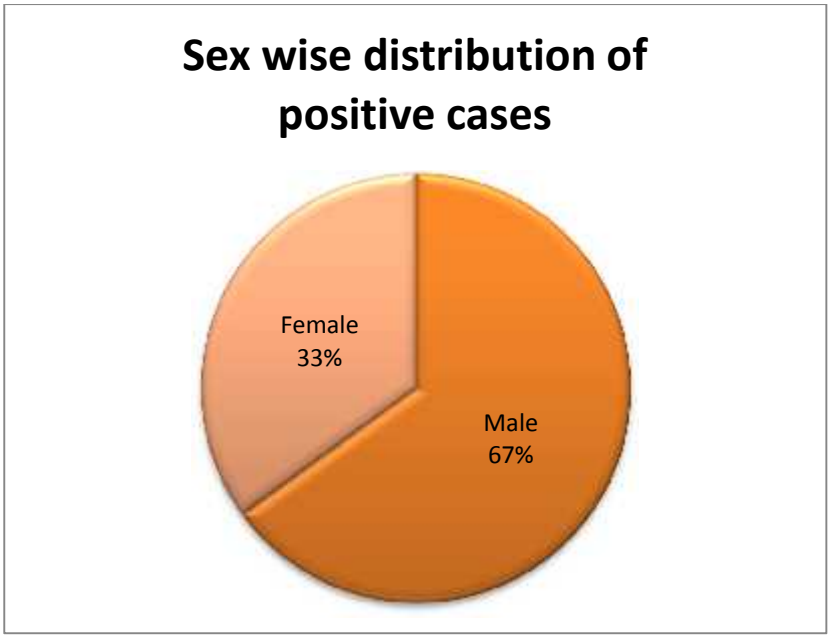


Table 15:Mode of dialysis of positive cases

Mode of dialysis	NUMBER(n= 9)
Peritoneal dialysis only	0
Hemodialysis only	6(67 %)
PD then HD	3(33%)

The above table :15 showed that among HCV infected cases,about 67% were under HD group and 33% were from peritoneal dialysis initially followed by hemodialysis. None of the cases were infected from peritoneal dialysis alone in infected group .P value was derived and significant statistically representing hemodialysis patients have higher positivity rate.

Fig:7 Mode of dialysis of positive cases

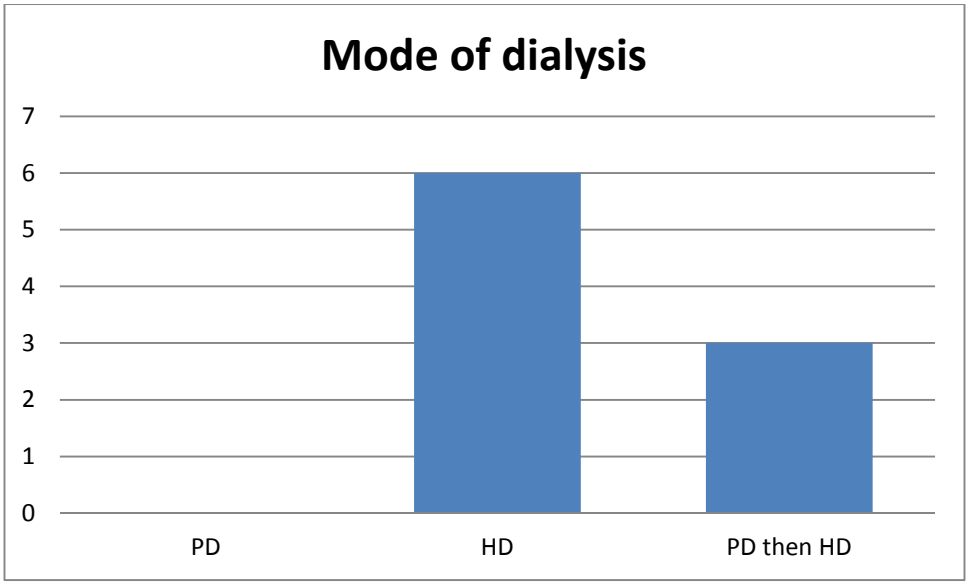


Table-16. Association of duration of dialysis of HCV positive cases

Duration of dialysis(months)	HCV positive Cases n=9		HCV negative Cases n=91	
	No	%	No	%
5-10	1	11	58	64
11-15	0	0	10	11
16-20	1	11	12	13
21-25	3	33	8	9
26-30	3	33	1	1
>30	1	11	2	2
TOTAL	9		91	
Mean duration	23.44±7.09		11.47±7.06	

The above table:16 showed that 7 (77%)cases among HCV positive group had duration of dialysis of more than 20 months. Among HCV noninfected cases only 12% had longer duration of dialysis. The mean average duration of dialysis in infected cases was about 23 months. The mean duration of dialysis in non infected cases was 11 months .The difference between them was 12 months. Association of duration of dialysis and HCV positivity had significance statistically ($P<0.05$) by students t test.

Fig 8:Association of duration of dialysis of HCV positive cases:

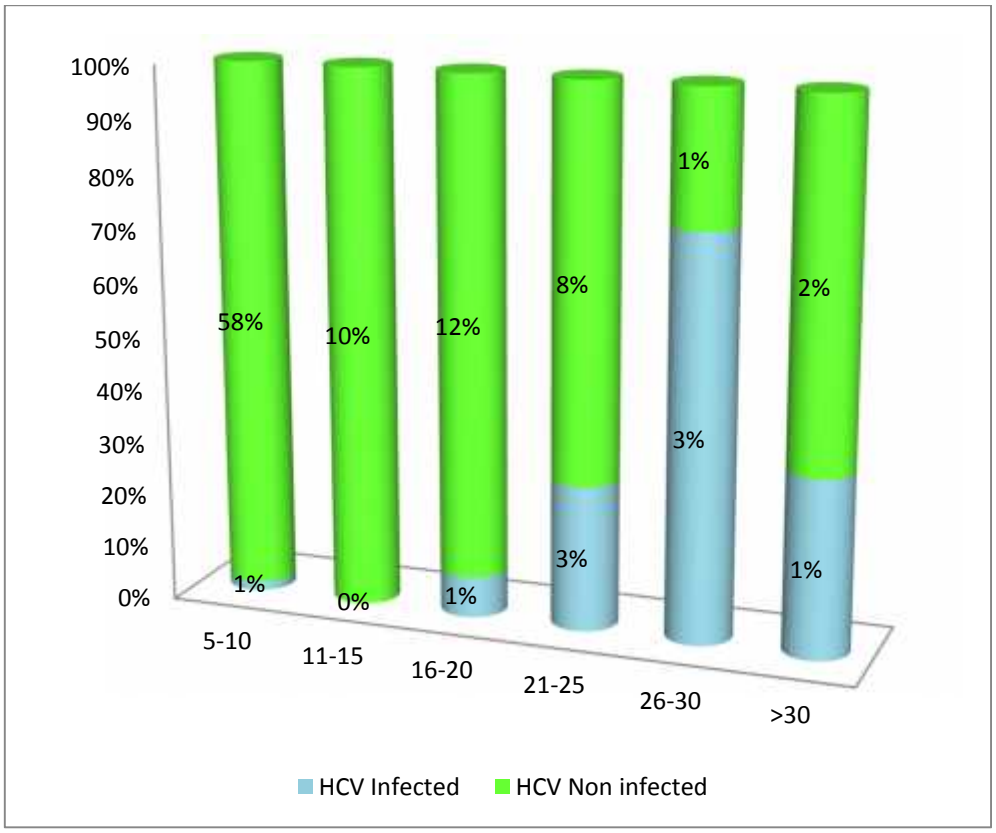


Table 17. Association of blood transfusions in HCV positive cases :

No of Blood transfusions	HCV positive Cases n=9		HCV negative Cases n=91	
	No	%	No	%
Did not receive any transfusion	5	55	67	74
< 3 times	1	11	21	23
3-6 times	2	22	2	2
>6 times	1	11	1	1
Total	9		91	

The above table:17showed the association of blood transfusion in HCV positive and HCV negative cases. Among positive group,1 (11%) case had blood tansfusion more than 6 times ,2 cases (22%) had transfusion history of 3-6 times, one (11%) case had transfusion of less than 3 times and 5 cases(55%) never had transfusion . Among negative group,23(26%) cases had multiple blood transfusion of more than 3 times.One case had transfusion of more than 6 times .67 cases (74%) never had transfusion .

Association of number of blood transfusion and HCV positivity was not statistically significant($P<0.05$)

Fig 9 : Association of blood transfusions in HCV positive cases

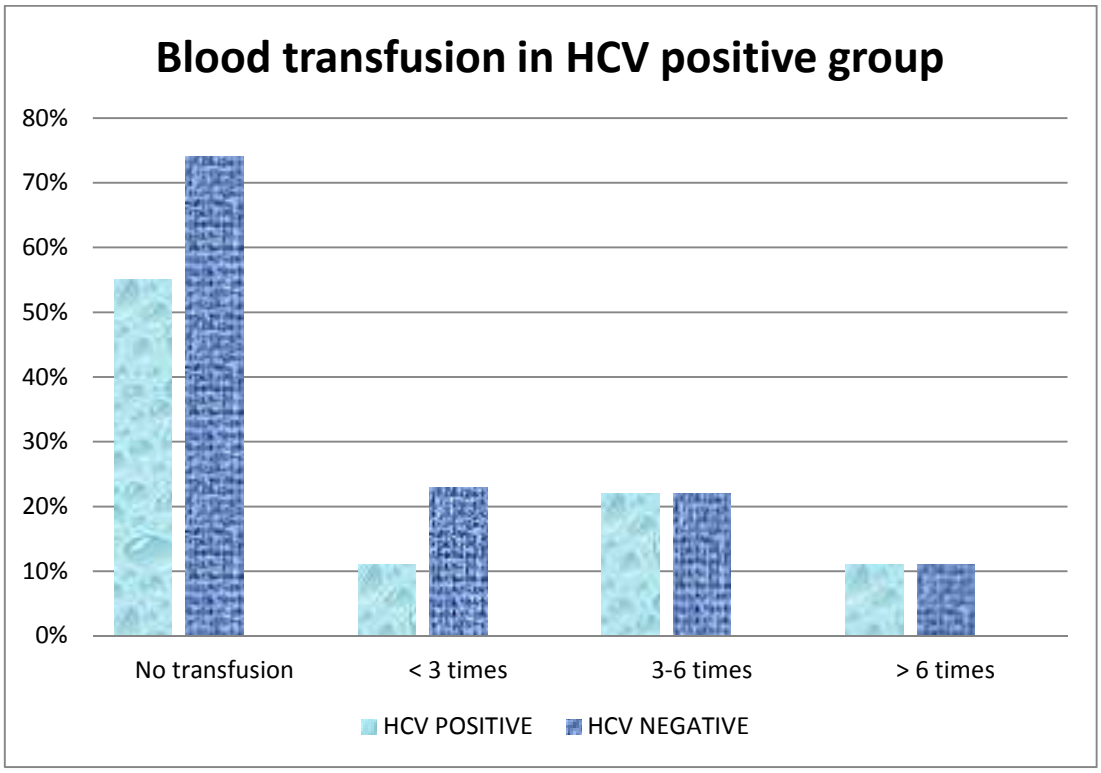


Table 18. Correlation of multiple dialysis centres visit with HCV positivity

No of dialysis centre visit	HCV positive cases n=9	HCV negative Cases n=91
1	3	68
2	3	20
3	2	2
4	1	1

Among the infected group in this study ,six cases had dialysis in multiple centres and three cases underwent dialysis in a single centre. Among non-infected group, 68 cases had dialysis in a single centre ,23 cases had dialysis in multiple centres. P value was statistically significant for number of dialysis centres and positivity of HCV .

Fig 10 Correlation of multiple dialysis centre visit with HCV positivity

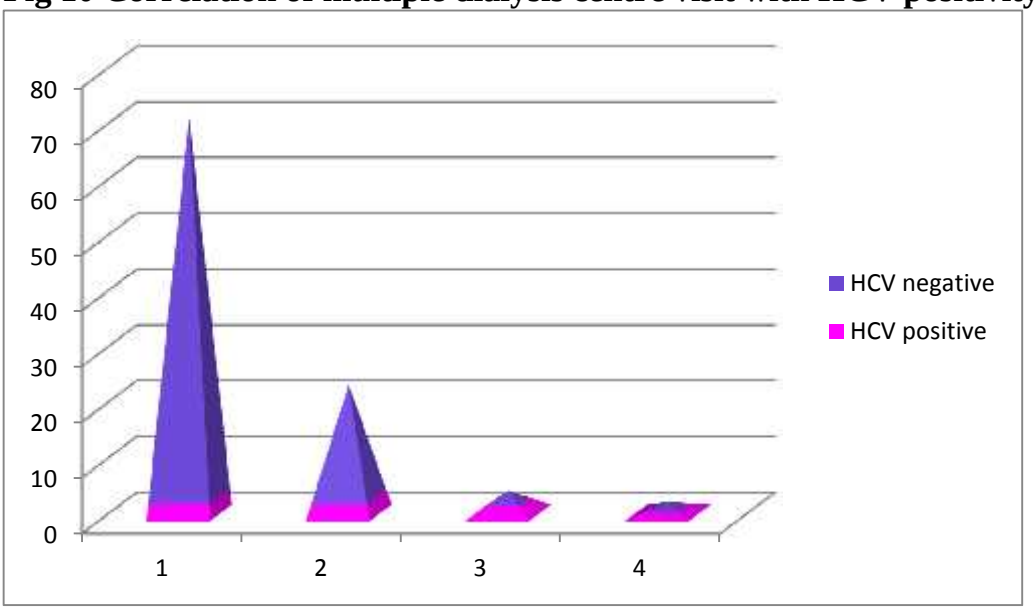


Table 19:Association of vascular access procedure with HCV positivity

No of times vascular procedures performed	HCV positive Cases n=9		HCV negative Cases n=91	
	No	%	No	%
0	0	0	13	14
1	2	22	48	53
2	4	45	29	32
3	3	33	1	1
Total	9		91	

The above table showed the correlation of vascular access procedure performed in multiple times had significance in positivity of HCV. Among positive cases ,7 (78%) cases had more than once vascular access done. In HCV negative group,30 cases (33%) had more than once vascular access performed. P value was significant in showing positive association between vascular procedure and positivity rate(<0.05) and is determined by Chi-square test.

Table 20:Association of organ transplantation among HCV positive cases

KIDNEY TRANSPLANT	HCV positive cases n=9	HCV negative Cases n=91
YES	1	1
NO	8	90

The above table showed that one case had renal transplantation done on positivity cases and one case had renal transplantation done on negativity group. There was no significant association between HCV positivity and organ transplantation in this study.

Table 21: Correlation of serum ALT levels with HCV positivity

ALT LEVEL	HCV Positiven=9		HCV Negativen=91	
	No	%	No	%
LOW NL	4	44%	16	18%
HIGH NL	4	44%	69	76%
ELEVATED	1	11%	6	6%

The above table:21 showed that serum ALT levels were within normal range for 8 cases(88%) in HCV positive group .Among the HCV negative group 85

cases(94%) had normal ALT levels. There was no significant association between serum ALT levels and positivity rate..P value was not statistically significant by Chi-square test.

Fig 11:Correlation of serum ALT in HCV positive group

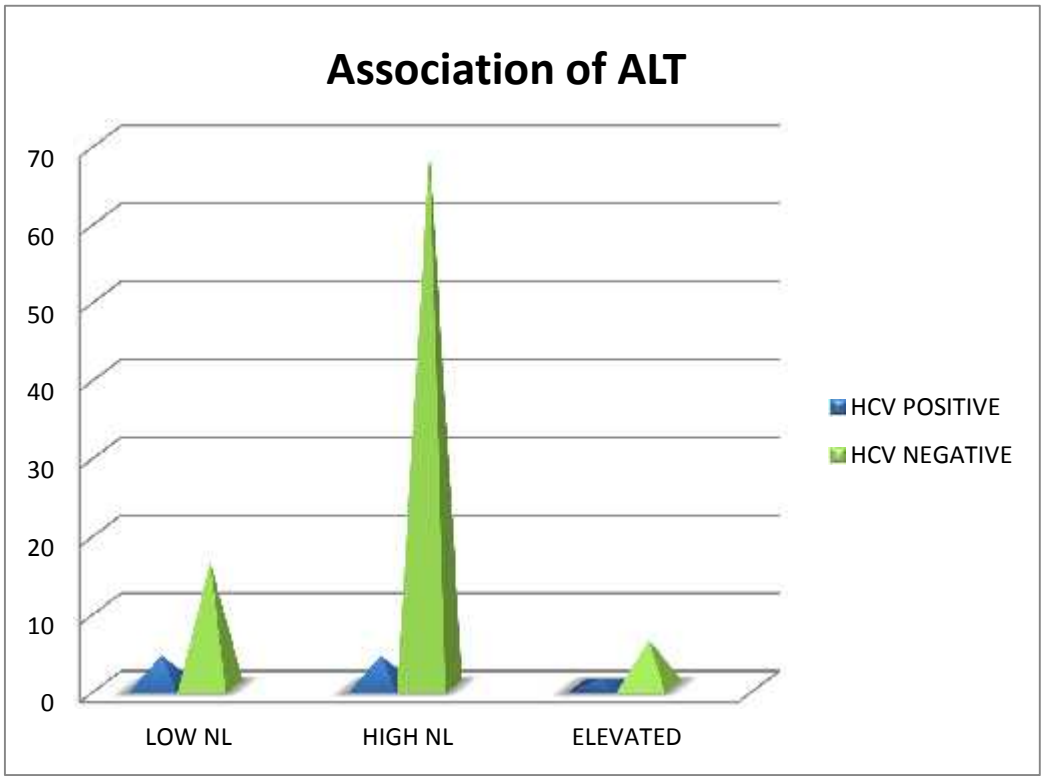


Table-22 :Association of other risk factorswith HCV positivity

RISK FACTORS	HCV positive Cases	HCV negative Cases
H/o surgery	1	4
H/o tattooing	0	9
Shaving inCommunity barber shops	2	33
Previous H/o jaundice	3	6
H/o blood contact	1	5
Drug abuse	0	0

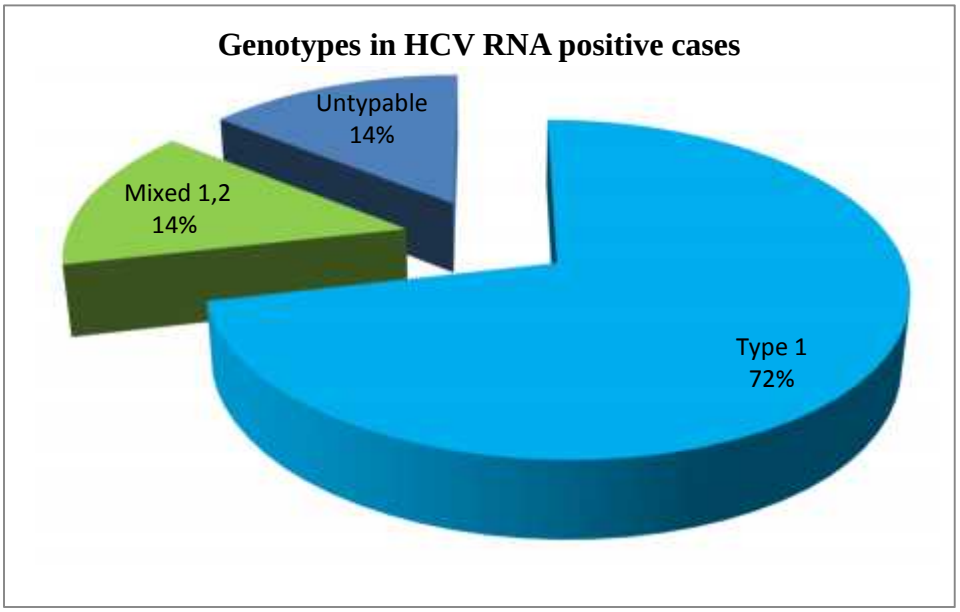
The above table:22 showed the various other risk factors in HCV transmission .Among the positive cases one case had history of surgery ,3 cases had jaundice history, one case had multiple dental extraction procedure done,2 patients had history of shaving in community barber shops and body piercing procedure. None of the positive cases gave history of drug abuse or tattooing .Two positive cases had no other known risk factors.

Table 23 :Distribution of genotypes in PCR positive cases

GENOTYPES	PCR POSITIVE cases(n=7)	PERCENTAGE
Type 1	5	72
Mixed(1and 2)	1	14
Untypable	1	14

The above table:23 showed the HCV genotypes prevalence in the positive cases.Five cases(72%)had type 1 ,one case(14%) had mixed genotype of 1 and 2.One case(14%) had untypable genotype .None of the cases had genotypes 3,4,5 or 6 in the current study.

Fig 12:Genotypes in PCR positive cases



6.DISCUSSION

Chronic kidney disease (CKD) was included among non –communicable diseases(NCD)in 2011by World Health Organization. HCV is linked to CKD in several ways –some forms of renal disease are precipitated by HCV and ESRD patients are at rising risk of HCV acquisition. Nowadays, the spread of HCV among dialysis patients is declining but its prevalence remains high mainly in developing countries. This study documented the HCV prevalence among CKD patients .Periodic screening of HCV infection is mandatory to prevent newer transmission and there is a need of effective diagnostic method in high resource regions. With this back-ground, this study entails in demonstrating the efficiency of ICT, ELISA in comparison with Real time PCR in detection of HCV .This is one of the first study in identifying the HCV genotypes prevalent in this area and may act as an aid in designing therapeutic strategies in order to cure liver damage and reduce extra-hepatic complications of HCV, including progression of CKD to late stages in near future.

6.1 Age-sex wise distribution among study group:

In the present study of 100 chronic kidney disease patients on dialysis ,males were predominant than females in the ratio of 1.85:1.Male predominance was also observed in similar studies conducted by Khasia anwar et al [106] Lahore among dialysis patients with high male to female ratio 2.5:1.In a similar study conducted in

Calicut by Shabana Razmin et al [107] revealed around 83% of males and 17% of females among dialysis group .

Male predominance was mainly due to their exposure to various risk factors like tattooing ,blood donations, community participation .

In this study regarding the age of patients undergoing renal replacement therapy ,majority (63%) of the patients were in the age group of 31-40 yrs of age. The mean age in the present study was 39.4 years.

Similar findings were observed in studies done by Murthy et al[51] in Vellore and by Razmin et al[107] reported mean age of study group around 34 years and 42 years respectively and most affected age group 31-50 years.

Middle age group are mainly responsible for economic productivity of the family. If they are treated ,it will improve the economy of family.

6.2 Mode of dialysis among positive cases:

In the present study with 100 CKD patients, the total infected cases were 9 and all were on hemodialysis .None of the cases had HCV infection in patients with peritoneal dialysis alone.

Similar results showing higher prevalence of HCV in hemodialysis(HD) patients compared to peritoneal dialysis (PD) reported by Chan et al [108], Besso et al[109],Conway et al[110].

In contrast ,Wang et al[111] and Huang et al [112] had reported equal prevalence of HCV infection at about 10%-15% in Taiwan for both HD and PD patients.

In a study done by Johnson et al in Thailand showed the prevalence of HCV infection is higher in PD patients compared to HD [71].

The reason for higher prevalence in HD is mainly due to prolonged vascular access, increased frequency of blood transfusions and hospitalization, multiple invasive procedures ,nosocomial transmission via contaminated dialysis equipments and their co-morbid condition.

Lower prevalence of HCV in PD patients is mainly due to lower requirement of blood transfusion, absence of a vascular access site, domociliary location of therapy, less number of visits to hospital.

6.3 Distribution of various etiologies leading to CKD among study group:

In the current study, hypertension(HTN) accounted for half of the cases in study group (51%) followed by Diabetes mellietus(DM) 24%and chronic glomerulonephritis (13%).Other causes include congenital conditions like(vesicoureteric reflex, posterior urethral valve) ,obstructive causes(calculi, tumour causing hydro uretero nephrosis) and chronic interstitial nephritis .

Similar results had reported by Asima banu et al constituting hypertensive nephropathy(83 %) as a leading causing ESRD[46].

In contrast ,a study by Fabrizio Fabrizi et al in California reported Nephrangiosclerosis as the main etiology leading to renal failure.[36]

In another study by Duong et al [11]and Chigrupati et al[47] had reported equal incidence of hypertension and diabetes mellietus leading to ESRD among dialysis.

In a Taiwanese study by Lee et al reported ,Diabetes mellietus is the primary cause contributing to end stage renal disease ESRD[113].

Hypertension and CKD relationship is cyclical. Elevated blood pressure (BP)cause damage to the blood vessels within the kidney leading to impairment in filtration of fluid and waste which in turn leads to increased fluid volume in the blood causing increased BP.

Hyperglycemia causes activation of protein kinase and release of cytokines, leading to cellular hypertrophy, collagen synthesis and vascular changes. This is the pathogenesis of diabetic nephropathy which in turn causes CKD.HTN and DM are more widely prevalent in our population and so contributes largely to CKD .

6.4 Comparison of rapid ICT, ELISA and RT-PCR in detection of HCV:

The positivity rate of rapid ICT was 7% and that of Elisa was 8%.The positivity rate of RT-PCR was 7%. Over all positivity rate and prevalence of HCV infection was 9 % in the current study.

Similar results was reported by Mohammad Ali Assarehzadegan et al [75] in 2009 that the prevalence rate of HCV was 7.9%.Similar results were obtained by Kanagapriya et al[52] in which seroprevalence was5.8% in Tirunelveli in 2011 out of 121 patients on HD. Kumar *et al*[53] (2011) reported 12.4%. of HCV prevalence in hemodialysis patients in Coimbatore which was similar to this study.

In contrast very high prevalence of 34.6% and 45% was reported from an Jordanian and Syrian study done by Salma Bdour et al[90] and Abdul Karim et al respectively[114]

.Avery high prevalence rate of HCV reported as 76 % in Moroccan study by Fabrizi et al in 2005 [36].

An Indian study by Jaiswal et al reported the prevalence of HCV infection was 30 % among dialysis patients [55] .A n Indian study from Bangalore by Asima Banu et al [46] reported very low percentage of prevalence of about 0.02%

Geographical location, socio economic factors and health care procedure related to adoption of universal precaution policy ,dialysis unit hygiene, isolation of HD machine ,proper sterilization of equipments and dialyser reuse are the factors which influence the prevalence of HCV.

Several studies reported HCV RNA positive with anti-HCV negativity among dialysis patients varies from 0%-12%[115].

There was some variation between serologic assay and virologic assay in the present study also. About 1 % of patients were anti-HCV negative but had HCV RNA detected by RT-PCR. The reason was mainly due to the fact that these uremic patients are in partial state of immunosuppression and failed to mount an efficient antibody mediated immune response to viral antigen and dysfunction in cell mediated immunity .Also the expression of antigen and antibody in these immunocompromised patients was very inconsistent, irregular and fluctuating.

Similar results was reported in a Netherlands study in which false negative rate for serology is 2/2286 accounting for 0.1%[78] .In a study by Yen et al [96] and Stradder et al[97] in California reported higher HCV RNA – positive with anti-HCV negativity of 153 (1.66%). Similar results of small significant proportion of HCV

RNA positive with anti-HCV negative serology was also observed in studies done by Hanuka et al [116] (9%) and Hinrichsen et al(0.8%)[38] .

A study by Pawlotsky et al[117] and Al Meshari et al [118] reported the HCV RNA positivity among anti-HCV negative cases in dialysis population and they concluded that the window period was longer in these patients.

In contrast to present study ,Zero false negative rate for serology was reported by Garinis et al[119],Sullivan et al in Athens[120]and Bouzgarrou et al[121] in Tunisia .

In the present study, out of total positive of 9 cases,2 cases were positive for anti-HCV serology by Elisa but negative for HCV RNA detected by Real time –PCR. The possible explanations related to this may be either due to past resolved infection or patients may be in the state of intermittent viraemia or occult infection .

A study by Salama et al in France reported the anti-HCV positivity was 16% while that of HCV RNA detection was 12% in hemodialysis patients [122]

Similar results was reported in Mexico by Mendez-Sanchez in which anti-HCV(3.0 Elisa) positivity was 6.7% and HCV RNA positivity(RT-PCR). was 5.4%[123].

A study by Fabrizi et al reported that 33% -67% cases of anti-HCV positive cases have intermittent viraemic state[83].

In contrast to the current study,Garinis et al reported that the anti-HCV positivity correlated with that of HCV RNA by RT-PCR in a Greece study in 1999[119].

6.5 Evaluation of Elisa with Real time PCR:

In the current study, the sensitivity of third generation Elisa was 85.7% when evaluated against RT-PCR, a reference test. Specificity of Elisa was 97.85%compared to RT-PCR. The positive predictive value and negative predictive value were 75% and 98.9% respectively.

Similar report was given by Prakash et al [41] in Lucknow in 2014 where the sensitivity and specificity of Elisa was 80% and 97.78% whereas the positive and negative predictive values were 76.19 %and 98.21% respectively.

In a study by Jasuja et al [40] revealed the sensitivity and specificity of anti-HCV Elisa was 73% and 98% respectively against RT-PCR .This study has lower sensitivity compared to the present study but specificity remains the same.

An Israelian study reported by Wienstein et al detected the sensitivity of 3.0 Elisa was little higher than the current study and was 94% and specificity was 91%.The positive predictive value was 76%[124] .

The kappa value agreement between ELISA and PCR was 79% in this study. Similar results were obtained by a Moreira et al in which the kappa value agreement between them was 71% [125] .The kappa value agreement between two tests was 0.61 in a study by Neerja Jindal[42].

ELISA is used as a screening test for serological detection of anti-HCV for its sensitivity and ease of performance .But in dialysis patients ,due to poor immunological reactivity ,adequate antibodies are not formed. This leads to false negative ELISA and hence there is a need for molecular detection methods.

6.6 Evaluation of ICT with RT-PCR and ELISA:

In the present study, sensitivity of rapid ICT was 71%,specificity 98 %,positive predictive value 71% and negative predictive value 98% in comparison with RT-PCR ,a gold standard test.

A study done by Ali et al reported 100% concordance results among dialysis group patients tested byrapid ICT and RT-PCR test. This is in contrast to our study[126].

A study from Lahore in 2010 by Khan et al [127] reported low senstitivity of rapid ICT test. The sensitivity and negative predictive values were very low and were 66% and 43% respectively. A Pakistanian study in 2012 by Hayder et al reported the sensitivity ,specificity, positive predictive value, negative predictive value of rapid ICT were 86 %,95 %,96%,87 % respectively. This value is higher than the present study**[128].**

In the present study, there was only a small difference between ICT and Elisa test results, that of 7% and 8% positivity respectively.

Similar to present study, a study demonstrated small differences between ICT and Elisa technique results for anti-HCV (1% and 3% positivity respectively) by Tajeldin et al[91] in 2015.

ICT is a rapid test with low sensitivity. It can be used in low resource setting as a screening tool. But needs further confirmation with ELISA or RIBA or molecular methods.

6.7 Age-sex wise distribution among HCV positive cases:

In the present study, males constitute most of the positive cases compared to females in the ratio of 2:1 .

Similar findings was studied by Nayle Maria Oliveria da Silva et al [74] in Southern Brazil that males 63% were infected with HCV in comparison to females.

A study by Muhammud et al [129] from Pakistan and Hegde et al [45] in Mangalore also observed the prevalence of HCV infection to be more in males.

This study was in similar agreement to the fact that male population is affected more in comparison to females. This is contributed mainly by exposure status to varying risk factors due to their life style and due to their cultural attributes . Also one study reports the clearance of HCV virus in females more due to the effect of estrogen hormones.

In contrast, Chopra et al [17] quoted that there is neither sex nor ethnic group difference found to be correlated with HCV infection independently.

In contrast to the present study, there is higher prevalence of HCV infection in females constituting 53% among dialysis patients in a study by Jia –Jung Lee in 2014[113] .

Regarding age in this study ,half of the infected case belongs to 31-40 year age group. This age group of high HCV reactivity was also reported by Kranti Kosaraju et al at Manipal in 2013 [130].

Saxena et al[131] and Karkar et al[132] studied and reported the increased vulnerability of HCV towards middle aged and elderly age group compared to younger age group.(< 24 years).

The mean age of males in the present study was 39.4 years and that of females same of 39.4 years. Over all mean age was 39.4 years.

Similar results were obtained by Ummate et al[133] in Nigeria in2014 reported the mean age of study group was 39.9 years.

*B*haumik et al [134]reported the age group between 40-60 years is significant among both study and infected cases group in 2012 in Tripura.

In contrast to present study, a study by Prasad KP Babu et al reported the higher prevalence of HCV in the age group of 60 -70 years among dialysis patients[135].

Middle age and elderly preponderance is mainly related to the additive effect of immunosuppresion along with advancing age, malnutrition, uremia. Increasing age contributes to HCV prevalence more because of the continuing risk of exposure.

Usually there is a lesser prevalence of HCV infectivity in age less than 20 years and is mainly due to strong immunity in younger age group(age related immunity).

6.8 Association of duration, frequency and number of dialysis among study group:

In the current study, HCV infected cases had longer duration of dialysis treatment compared to non infected cases. The mean duration of dialysis of infected cases was about 23 months. 66 % of infected cases had dialysis duration of21 -30 months .

Similar results were obtained by Chawla et al[13], Dussol et al [136], Zacks et al[137] in which duration of dialysis was reported as one of the major contributing factor HCV infection. In a study by Chawla the mean duration of dialysis was 8 months in HCV positive cases.

HCV positivity for patients on dialysis for more than 3 years was 94.5% compared to patients on dialysis for less than 1 year (16.4%) in a study by Al-Jiffiri et al in 2003[138].

Mean duration of dialysis in HCV positive cases was 3.6 years compared to negative cases in which mean duration was 2 years in a study from Mangalore by Hegde et al [45]. Mean hemodialysis duration was 148.1 months among HCV positive cases which was very high reported by Moreira et al.[125]

All the risk factors-duration, frequency and number of hemodialysis were related to nosocomial transmission as well as dissemination of HCV infection within the dialysis units and extracorporeal circulation in these units. Environment itself acts as a vehicle in HCV transmission in dialysis centres. With increased dialytic duration , there is increased chance of exposure of patients to hospital environment frequently and inter personal spread is more likely. This may be due to contaminated multidose vial of heparin, usage of same gloves in between patients and increased parenteral interventions.

The present study also reported that increased number of hemodialysis sessions was significantly associated with increased HCV positivity.67 % of the HCV

positive patients in this study had number of dialysis sessions above 110.The mean number of sessions among positive cases was 122.

A study by Khasia Anwar et al in 2016 reported the significant association of frequency of dialysis and increased number of sessions in relation to HCV positivity[106].

The mean number of hemodialysis sessions was 391 and was correlated significantly with HCV positivity reported by a Vietnamese study studied in 113 patients on dialysis.[11]

In the current study 88% of positive cases had frequency of dialysis of twice a week and 12 % had thrice a week dialytic sessions .

PD patients undergo dialysis either every month or once in every three months depending on their general condition and uremic status .All the PD patients in this study were having intermittent dialysis only except one who was on Continuous ambulatory peritoneal dialysis.(CAPD).

6.9 Association of blood transfusion with HCV positivity:

In the current study, out of 9 infected cases, one case had multiple transfusion of more than six times. 2 more had blood transfusions three to six times and one case had transfusion less than 3 times and 5positive cases never had a transfusion. Among non infected cases ,one case had higher number of transfusions of more than 6 times and 67 cases never had any transfusion. There is no significant association between HCV positivity and blood transfusion in this study. Reduction of blood

transfusion requirements to prevent anemia was mainly with greater utilization of erythropoiesis-stimulating agents like Erythropoietin .

In 2003,Al-Jiffiri et al reported significant association of blood transfusion with HCV positivity inpatients .Out of 248 patients on dialysis ,75.8% of HCV positive patients had transfusion history[138].

Das et al [44] in 2015 from Ludhiana reported that there is no significant association between HCV reactivity and blood transfusion .This study was similar to present study.

In contrast, positive correlation between HCV positivity and blood transfusion was reported by Moreira et al[125] and Al Jamal et al[139] in 2005 and 2009 from Brazil and Jordan respectively in hemodialysis patients. Syspa et al in 2005[140] , Alashek et al [141] in 2012 reported that increased number of blood transfusions cause more HCV positivity.

6.10 Association of dialysis to multiple centres with HCV positivity :

Among the infected group in this study ,six cases had dialysis in multiple centres and three cases underwent dialysis in a single centre. Among non-infected group, 68 cases had dialysis in a single centre ,23 cases had dialysis in multiple centres P value was statistically significant and there was a correlation between number of dialysis centres and HCV positivity.

In studies by Alashek et al in 2012 from Libya[141] ,, and Duong et al in Vietnam[11], there was a positive significant association between multiple dialysis centres and increased HCV prevalence .In a study by Kumar et al in 2011 from

Coimbatore, the visit to multiple dialysis units was a major risk factor associated with HCV positivity[53] .

6.11 Association of organ transplantation and other risk factors among positive group :

Among the infected, one case had renal transplantation done Similarly among HCV negative cases ,one case had undergone renal transplantation .There is no significant correlation between organ transplantation and HCV positivity.

Similar result was obtained by Hegde et al in Mangalore[45] who found out insignificant association between HCV positivity and organ transplantation

In contrast to this study, Schneeberger et al[78] , Salama et al [122], Syspa et al [140] all reported that there is significant positive association between organ transplantation and HCV positivity.

Proper effective screening of donors will reduce HCV transmission rates.

6.12 Association of ALT levels in relation to HCV positivity :

In the current study ,among HCV positive case**s** , 88% had ALT levels within normal limits.12% had elevated ALT levels .No significant association was found between HCV positivity and serum ALT levels.

Studies by Hanuka et al [116],Kumar et al [53], Chawla et al [13] were similar to the current study denoting that there is no significant association statistically between serum ALT level and HCV positivity. Reduced ALT levels may be due to hemodilution, suppression of ALT synthesis by hepatocytes .

In contrast to the present study, Kosaraju et al[130] and Vikkas Makkar et al[43] in 2014 from Ludhiana showed that HCV positive cases had higher mean value of ALT levels compared to controls and was significant .This was probably due to the destruction of hepatocytes caused by body's immune reaction in response to hepatitis viruses leading on to increased release of aminotransferases.

6.13 Association of other risk factors with HCV positivity

None of the patient had drug abuse in this study.

History of drug abuse was a risk factor in HCV prevalence in a study by Vikkas Makkar et al[43] .

All patients had dialyser reuse and the dialyser was used for the same patient after proper disinfection for upto 8 times.No significant impact on dialyser re-use was noted in this study.

6.14 Genotypes prevalent in PCR positive cases

In the present study ,most prevalent genotype is type 1 accounting for 72% cases.14% had mixed genotypes(type 1,2) and another 14 % had untypable genotypes.

Similar results were observed in a Japanian study[143] and Saudi Arabian study [144] in which genotype 1 was the most prevalent (74 %)among dialysis population.[1,2] Genotype 1 was most prevalent among dialysis patients in an Israelian study by Wienstein et al [124] and in a Brazilian study by Vidales –Braz[37] in the year 2001 and 2015 respectively.

Untypable isolates accounted for 15.6% in Pakistanian studies when routine diagnostic methods were used . Waqar *et al*[145] in 2014 recognised 66 (12%)and Ali *et al* in 2014 [146] observed 21 (17.35%) untypable variants .

Probably untypability was due to low viral load leading to less capability of genotype assessment . Hence there is a need for most reliable testing by direct sequencing for knowing the nature of genotype(recombinant). Error prone nature of viral Polymerase in HCV genome has mutation rate of approximately 10^{-3} per nucleotide per replication leading to quasi- species .This changing pattern is multi-factorial and might be due to high viral genome mutation rate ,host immunological pressure, drug pressure ,viral or host immune escape mechanism, changes in transmission route, migration to other areas and several other unknown factors[146].

Mixed genotype accounted for 5 cases(12%) out of 40 HCV RNA postitive cases in a study by Hairul Aini et al in 2012 [147] stated that all chronic dialysis patients had an equal chance of getting single or mixed genotype infection .No risk factors were associated with mixed genotype in the study.

Mixed genotypes were prevalent in 5.5% cases in a Pakistanian study by Sadia Butt et al.[148]

Qian *et al.*, in 2000 [149] reported the parenteral route of transmission in some cases of mixed genotype.

Mixed genotype have been associated with immune escape mechanism leading to chronicity and severe disease progression .In mixed genotype cases ,change in predominant genotype occurred over time due to immune selection pressure.

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7.SUMMARY

The present study aimed at detecting the prevalence of HCV antibody ,HCV viraemia in serum samples of adult chronic kidney disease patients on dialysis . 100 cases of adult CKD patients were included in the study and samples were tested by rapid Immunochromatographic test and ELISA for anti-HCV .All the samples were subjected to Real-time PCR for HCV RNA isolation Genotyping was done for all PCR positive cases by PCR with type labeled primers .Various factors associated with dialysis patients were analyzed.

- Of the 100 samples tested , majority of cases 63 % were in fourth decade.
- Out of 100 cases,65 % were male and 35 % were female in the study group.
- The most common causes leading to chronic kidney disease among the study group were Hypertension (51%), Diabetes mellitus (24%),chronic glomerulonephritis(13 %)
- 87 % of cases were on Hemodialysis and 13 % were on Peritoneal dialysis in the study group.
- Out of 100 adults tested by rapid ICT, 7% had anti-HCV antibody in the serum sample.
- When the same samples were tested by ELISA, positivity seen in 8% of samples.
- 7 % samples positive by ICT were positive by ELISA while 1% sample negative by ICT was positive by ELISA.

- All the same samples were subjected to Real-time PCR, HCV RNA was detected in 7 % of cases.
- 6 % samples positive by ELISA were positive by RT-PCR also.
- 1 % sample negative for EIA had positivity by RT-PCR ; whereas 2 % samples had ELISA positive but RT-PCR negative .
- Overall infectivity rate of HCV was 9 %
- Rapid ICT had a sensitivity of 71.4%, Specificity of 97.8% for anti-HCV antibody when evaluated against Real-time PCR, as gold standard.
- ELISA had a sensitivity of 85.7%.Specificity of 97.5 % for anti-HCV when evaluated against Real-time PCR ,as gold standard.
- All infected cases (100%) were undergoing hemodialysis.
- Maximum number of positive cases (44%) occurred in fourth decade of life with male preponderance(67%)
- Risk factors are longer duration of dialysis and multiple dialysis centre visit was found to be associated with 77 % and 67% of positive cases.
- Genotype 1 was presented in 72 % of positive cases .Mixed genotype (Type-1,2) was seen in 14% of positive cases and another 14 % of positive cases had untypable genotype.. No case presented with genotypes 3,4,5,6 in the current study.

8.CONCLUSION

- The current study determines HCV infection prevalence in chronic kidney disease patients on dialysis.
- Rapid Immuno chromatographic test is easy to perform and interpret in low resource settings. This test is less sensitive and needs further confirmation.
- ELISA test is considered as a diagnostic tool for anti-HCV detection because of its ease of use ,cost effectiveness and low variability. False negative ELISA is due to poor immunological reactivity in these patients.
- PCR is the "gold standard" for RNA detection of Hepatitis C in patients undergoing dialysis .But PCR may be negative in ELISA positive cases due to fluctuating viraemia.
- The best way for screening dialysis patients is to combine immune-enzymatic method and molecular method for synergistic effect in detecting HCV and this further helps in preventing HCV transmission.
- Stringent adherence to universal precautions, isolation of hemodialysis machines, ideal nursing practices and proper sterilization of equipments and periodical viral parameters monitoring are essential in reducing HCV transmission.
- Genotyping of patients on dialysis may be helpful in formulating prevention and treatment strategies.

BIBLIOGRAPHY :

1.Textbook of clinical microbiology Patrick Murray Hepatitis C and G viruses chapter 93 ,John D.Scott ,David R.Gretch pages 1437-1447.

2. Atreyi Chakraborty,Sampurna Biswas Pramanik,Debajyoti Singha Roy,Soma Sarkar,Mayukh Chakraborty and Anita Nandi , A retrospective study on the sero-prevalence of Hepatitis C Infection in a tertiary care hospital in Kolkata,India *International Journal Of Current Microbiology and Applied Sciences* (2015) 4(3):115-123.

3.Shah N,Kadla S,Chouhan I ,Ali I,Iqbal A,Parveen S, et al.Clinical ,Biochemical,Virological and Histopathological Profile Of Incidentally DetectedcHepatitis C ,*J Clin Exp Hepatol* ,4:S23-S4.

4. Smaragdi Marinaki, John N Boletis, Stratigoula Sakellariou, Ioanna K Delladetsima Hepatitis C in Hemodialysis *World J Hepatol* 2015 March 27; 7(3): 548-558

5.Khaja MN, SK Munpally, MM Hussain and CM Habeebullah. 2002 Hepatitis C virus: The Indian scenario; *Current Science Vol* 83, No 3,10 August 2002, 219-24.

6. Sanaullah Khan, Sobia Attaullah, Ijaz Ali, Sultan Ayaz, Naseemullah, Shahid Niaz Khan, Sami Siraj and Jabbar Khan Rising burden of Hepatitis C Virus in hemodialysis patients V*irology Journal 2011*, 8:4385.

7. Norberto Perico, Dario Cattaneo, Boris Bikbov, and Giuseppe Remuzzi Hepatitis C Infection and Chronic Renal Diseases *Clin J Am Soc Nephrol* 4: 207–220, 2009.

8.J Christdas, J Sivakumar, J David, HDJ Daniel, S Raghuraman, P AbrahamGenotypes of hepatitis C virus in the Indian sub-continent: A decade-long

experience from a tertiary care hospital in South *India IJMM 2013 vol 31 pages 349-353*.

9.Mohammad Rahnavardi a Seyed Mohammadmehdi Hosseini Moghaddam bSeyed Moayed Alavian Hepatitis C in Hemodialysis Patients:Current Global Magnitude, Natural History,Diagnostic Difficulties, and Preventive Measures *Am J Nephrol* 2008;28:628–640

10.A.A. Amir, R.A. Amir and S.S. *Sheikh* Hepatitis C Infection in Kidney Transplantion *INTECH Open science/Open minds*

11.Cuong Minh Duong, Dariusz Piotr Olszyna and Mary-Louise McLaws
Hepatitis B and C virus infections among patientswith end stage renal disease in a low-resourced hemodialysis center in Vietnam: a cross-sectionalstudy
BMC Public Health (2015) 15:192

12.Dalekos GN, Boumba DS, Katopodis K, Zervou E, Sferopoulos G, Elisaf M, *et al.* Absence of HCV viraemia in anti-HCV-negative haemodialysis patients. *Nephrol Dial Transplant 1998;13:1804-6.*

13. *NS Chawla, CT Sajiv, G Pawar, B* Pawar Hepatitis B and C Virus infections associated with renal replacement therapy in patients with end stage renal disease in a tertiary care hospital in India – prevalence, risk factors and outcome.
Indian J Nephrol 2005;15: 205-21315.

14 N. L. Latt , F. Araz , N. Alachkar , C. M. Durand , A. Gurakar
.Management of Hepatitis C infection among patients with renal failure.
Article in Minerva gastroenterologica e dietologica · 2015;61:39-49

15.Seyed-Moayed Alavian, Ali Kabir,Amir Bahrami Ahmadi,Kamran Bagheri Lankarani, Mohammad Ali Shahbabaie,Masoud Ahmadzad-ASL Hepatitis C infection in hemodialysis patients in Iran: A systematic *review Hemodialysis International 2010; 14:253–262*

16. Peter Simmonds The Origin of Hepatitis C Virus*Hepatitis C Virus: From Molecular Virology to Antiviral Therapy*, *Current Topics in Microbiology and Immunology 369*

17. Cheney CP, Chopra S, Graham C. Hepatitis C. *Infect Dis Clin N Am*. 2000;14(1):633-37.

18.David Greenwood,Will Irving,Mike Barer,Richard Slack Medical microbiology –A guide to microbial infections ; Eighteenth edition ; Hepaciviruses and Hepeviruses ,P.Simmonds : pages 537-544

19.Mandell Douglas and Bennett ,Principles and practices of Infectious diseases 7 th edition Hepatitis C chapter 156 ,Stanley Lemon : pages 1902-1927

20. Lindenbach, B.D. and C.M. Rice, Unravelling hepatitis C virus replication from genome to function. *Nature, 2005.* ***436****(7053): p. 933-8.*

21. Zhu, H., et al., Gene expression associated with interferon alfa antiviral activity in an

HCV replicon cell line. *Hepatology, 2003.* ***37****(5): p. 1180-8.*

22. Eriksen, M. Molecular and Epidemiological Profiles of Hepatitis C Virus Genotype 4 in Denmark. *Journal of Medical Virology2010*

23.Pang PS, Planet PJ, Glenn JS. The evolution of the major hepatitis C genotypes and correlates with clinical response to interferon therapy. *PLoS One 2009;4:e6579.*

24 . Dustin, L.B. and C.M. Rice, Flying under the radar: the immunobiology of hepatitis C. *Annual review of immunology, 2007.* ***25****: p. 71-99.*

25. World Health Organisation WHO fact sheet Hepatitis C updated July 2015

26.Sherlock's Diseases of the liver and biliary system ,*Twelfth edition,*Edited by James S.Dooley,Anna S.F.Lok,Andrew K.Burroughs,E.Jenny Heathcote chapter 20 Hepatitis C Geoffrey Dusheiko *pages 407-426.*

27. . Bowen, D.G. & Walker, C.M., 2005. Adaptive immune responses in acute and chronic

hepatitis C virus infection. *Nature*, 436(7053), pp.946–52.

28.Harrison's principles of internal medicine Kasper,Fauci,Hauser,Longo,Jameson,Loscalzo Vol-2 19 th edition Chapter 362 pages 2030-2045

29.Topley and Wilson's microbiology and microbial infections ,vol-1,Virology ,9th edition Hepatitis C Peter Simmonds ,David Multimer Pages 1-34

30.Sandeep Satsangi,Yogesh K Chawla Medicine update 2015- Hepatitis C :Suspect,detect and treat

31.Jawetz,Melnick,AdelbergMedicalMicrobiologyGeo.F.Brooks,Karen.C.Carroll,Janet.S.Butel,Stephen.A.Morse,Timothy.A.Mietzner 26 th edition pages 507-526

32. Liang T J, Rehermann B, Seeff L B and Hoofnagle J H 2000 Pathogenesis, natural history, treatment, and prevention of hepatitis C; *Ann. Int. Med.* **132** 296–305

33. Kidney Disease: Improving Global Outcomes (KDIGO).KDIGO clinical practice guidelines for the prevention,diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. *Kidney Int Suppl* 2008; (**109**).

34.Digdem Ozer Etik, Serkan Ocal, Ahmet Sedat Boyacioglu Hepatitis C infection in hemodialysis patients: A review *World J Hepatol* 2015 April 28; 7(6): 885-895

35.Zampieron A, Jayasekera H, Elseviers M, et al. European study on epidemiology and management of hepatitis C virus (HCV) infection in the hemodialysis population. Part 3: Prevalence and incidence. *EDTA-ERA J. 2006; 32:42–44*.

36.Fabrizio Fabrizi, Giovanna Lunghi, Sri Venkatesh Ganeshan, Paul Martin, and Piergiorgio Messa Hepatitis C Virus Infection and the Dialysis Patient *Seminars in Dialysis—Vol 20, No 5 (September–October) 2007 pp. 416–422*

37. Beatris Maria Vidales-Braz, Naylê Maria Oliveira da Silva, Rubens Lobato, Fabiana Nunes Germano,Luiza Dias da Mota, Elvino JG Barros *et al* Detection of hepatitis C virus in patients withterminal renal disease undergoing dialysis in southern Brazil: prevalence, risk factors,

genotypes, and viral load dynamics in hemodialysis patients :*Virology Journal (2015) 12:8*

38. H Hinrichsen, G Leimenstoll, G Stegen, H Schrader, U R Fölsch, W E Schmidt for the PHV study groupPrevalence and risk factors of hepatitis C virus infection

in haemodialysis patients: a multicentre study in 2796 patients *Gut 2002;51:429–433*

39. Haldun Selcuk · Mehmet Kanbay · Murat Korkmaz · Gurden Gur · Ali Akcay · Hande Arslan et al Distribution of HCV Genotypes in Patients with End-Stage Renal Disease According to Type of Dialysis Treatment ,*Dig Dis Sci (2006) 51:1420–1425*

40.S.Jasuja,A.K.Gupta,R.Choudhry,V.Kher,D.K.Aggarwal,A.Mishra,M.Agarwal,A.Sarin,M.K.Mishra,V.Raina Prevalence and associations of Hepatitis C viremia in hemodialysis patients at a tertiary care hospital,*Indian Journal Of Nephrology;April2009:vol 19/Issue 2*

41.Shantanu Prakash , S.N. Sankhwar , Amita Jain , Kausar Usman , Narayan Prasad , D. Saha *et al* Comparison of third generation ELISA and conventional nested RT-PCR for detection of HCV among hemodialysis patients *Journal of Applied Pharmaceutical Science Vol. 4 (08), pp. 018-022, August, 2014*

42. Neerja Jindal, Divya Soin, Pragati Grover, Renu Bansa, Rubina Malhotra, seema singh *et al* Hepatitis C Virus (HCV) Infection among Seronegative Patients undergoing Haemodialysis in a Remotely Located Tertiary Care Hospital of Northern India: Value of HCV-RNA and Genotypes /*JCDR/2015/15310.6952*

43. Vikas Makkar, Dinesh Gupta, Kanish Bansal, N. S. Khaira Prevalence, seroconversion and risk factors of Hepatitis B and C infection in patients on maintenance hemodialysis *J of evolution of med and dent sci/vol. 3/ issue 50/oct 06, 2014*

44. K.C. Das, Shavinder Singh, Sumeet David, Sanatan Behere and Saakhi Dugal A comparative study of the prevalence of and risk factors for hepatitis-B virus (HBV) and hepatitis-C (HCV) infections in patients of chronic renal failure undergoning renal replacement therapy- a prospecto-retrospective therapy *International Journal of Recent Scientific Research Research*

Vol. 6, Issue,4, pp.3651-3664,April, 2015

45. Hegde R and Boloor R Prevalence of Hepatitis c virus among the haemodialysis patients in a tertiary care centre and its association with risk factors *International Journal of Basic and Applied Medical Sciences issn: 2277-2103 (online) 2014 vol. 4 (2) May-August, pp. 146-148*

46. Asima Banu,Latha B,Asha Aravind,Prabhat Kumar A study on low sero-prevalence of Hepatitis C infection in Hemodialysis patients in South India *American Journal Of Advances In Nursing Research* ,2015*;2(2):58-61*

47.Pragati Chigrupati,S.Subbarayudu,Sarath Babu Study of incidence of Hepatitis C virus infection in hemodialysis patients ,*Journal of Dr.NTR University of Health Services 2014;3(1):19-22*

48. Reddy AK, Murthy KV & Lakshmi V. Prevalence of HCV infection in patients on haemodialysis: survey by antibody and core antigen detection; *Indian Journal of Medical Microbiology*. 2005

49. ShaliniAshokNaik, BanashankariG.S., Beena, GayathriDeviD.R., Sreeja Prevalence Of Hepatitis C Infection In A Tertiary Care Hospital In South India *NJLM July 2012 vol-1 pages :20-22*

50. Agarwal SK, Dash SC & Irshad M. Hepatitis C virus infection during haemodialysis in India; *J.Assoc.Physicians India.*1999;47:1139-43.2

51. KK Murthy, GT John, P Abraham, GS Talaulikar, PP Thomas, CK Jacob

. Profile of hepatitis B and hepatitis C virus infections in dialysis and renal transplant patients 1997-2001;CMCH Vellore *Indian J Nephrol 2003;13:24-28*

52.Kanagapriya M,Palaniappan N,Sucilathangam G Study of Sero prevalence of Hepatitis C viral infection in Hemodialysis patients in Tirunelveli *International Journal Of Scientific Research vol:2 /Issue :11/Nov.2013*

53. Surendra Kumar P, Venu G, Madhusudhana Rao A, Balakrishnan N, Saravanan T, Sofia Rani A and Subba Rao TM (2011). Prevalence and Risk Factors of Hepatitis C among Maintenance Haemodialysis Patients at a Tertiary-Care Hospital in Coimbatore, India. *Journal of Clinical Disease and Research* 5 725-28.

54. Douglas D.Richman,Richard J.Whitley, Frederick G.Hayden Clinical virology ,second edition .

55.Jaiswal SP, Chitnis DS, Salgia P, Sepaha A, Pandit CS. Prevalence of hepatitis viruses among chronic renal failure patients on hemodialysis in central India. *Dial Transplant 2002;31:234-8.*

56. . Neogi D K, Bhattacharya N, Chakrabarti T and Mukherjee K K 1997 HCV activity in Calcutta--a serological study; *J. Commun. Dis*. **29** 1–6

57. Rehan SH, Manak S, Yadav M, Deepinder, Chopra D, and Wardhan N.Diversity of Genotype and Mode of Spread of Hepatitis C Virus in Northern India. *Saudi* J *Gastroenterol. 2011; 17: 241–4.*

58.Saha M K, Chakrabarti S, Panda S, Naik T N, Manna B, Chatterjee A,Bhattacharya S K 2000 Prevalence of HCV& HBV infection amongst HIV seropositive intravenous drug users & their non-injecting wives in Manipur, India; *Indian J. Med. Res*. **111** 37–39

59. Mehta SH, Vogt SL, Srikrishnan AK, Vasudevan CK, Murugavel KG, Saravanan S et al. Epidemiology of hepatitis C virus infection & liver disease among injection drug users (IDUs) in Chennai, India. *Indian J Med Res. 2010 Dec;132:706-14*

60.Singh S, Dwivedi SN, Sood R, Wali JP. Hepatitis B, C and human immunodeficiency virus infections in multiply-injected kala-azar patients in Delhi. *Scand J Infect Dis 2000; 32: 3–6.*

61. Natov SN. Transmission of viral hepatitis by kidney transplantation: Donor evaluation and transplant policies(Part 2: Hepatitis C virus*). Transplant Infect Dis. 20024:124–131.;*

62 . Howard C.Thomas ,Stanley Lemon,Arie J.Zuckerman ,Viral hepatitis ,third edition pages 381-573

63. Kaldor JM,Archer GT,Buring ml et al rf of HCV in blood donors – a case control study *Med JAust 1992 ;157:227-230*

64.Khaja MN, Madhavi C, Thippavazzula R, Nafeesa F, Habib AM, Habibullah CM, et al. High prevalence of hepatitis C virus infection and genotype distribution among general population, blood donors and risk groups. *Infect Genet Evol. 2006 ;6(3):198-204*

65. Shepard WC, Finelli L, Alter JM. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis 2005; 5: 558–67*

66.Gowri V, Chandraleka C, Vanaja R. The current seroprevalence of Hepatitis C virus in a Tertiary Care Centre in Vellore, Tamil Nadu. *Indian J. Community Med. 2012; 37(2):96-110*

67. Bhattacharya S, Badrinath S, Hamide A, Sujatha S. Co-infection with hepatitis C virus and human immunodeficiency virus among patients with sexually transmitted diseases in Pondicherry, South India. *Indian J PatholMicrobiol. 2003 Jul;46(3):495-7*

68 .Koblin BA,Factor sh,Wu Y Vlahov D hcv among non injecting drug users in New York city *J.Medical Virology 2003;70:387-390*

69. Mohammad Javad Zahedi , Sodaif Darvish Moghaddam , Seyed Moayed Alavian , Mahdieh Dalili Seroprevalence of Hepatitis Viruses B, C, D and HIV Infection Among Hemodialysis Patients in Kerman Province, South-East Iran *Hepat Mon.2012;12(5):339-343*

70. Meyers CM, Seeff LB, Stehman-Breen CO, Hoofnagle JH (2003) Hepatitis C and renal disease: an update.*Am J Kidney Dis 42:631–635*

71. David W. Johnson,, Hannah Dent, Qiang Yao, Anders Tranaeus, Chiu-Chin Huang Dae-Suk HanFrequencies of hepatitis B and C infections among haemodialysisand peritoneal dialysis patients in Asia-Pacific countries: analysisof registry data *Nephrol Dial Transplant (2008) 1 of 5.*

72. Ahmetagic S, Muminhodzic K, Cickusic E, Stojic V, Petrovic J, Tihic N. Hepatitis C infection in risk groups. Bosn *J Basic Med Sci 2006;6:13-7.*

73. . Carneiro MA, Martins RM, Teles SA, Silva SA, Lopes CL, Cardoso DD, *et al.* Hepatitis C prevalence and risk factors in hemodialysis patients in Central Brazil: A survey by polymerase chain reaction. and serological methods. *Mem Inst Oswaldo Cruz 2001;96:765-9.*

74.Naylê Maria Oliveira da Silva, Fabiana Nunes Germano, Raul Andres Mendoza-Sassi Hector Nicolas Seuánez, Marcelo Alves Soares and Ana Maria Barral de *Martinez*

Evidence of association between hepatitis C virusgenotype 2b and nosocomial transmissions in hemodialysis centers from southern Brazil *Virology Journal 2013, 10:167*

75. Mohammad Ali Assarehzadegan, Ghodratollah Shakerinejad, Reza Noroozkohnejad,Akram Amini, S. A. Rahim Rezaee Prevalence of Hepatitis C and B Infection and HC V Genotypes among Hemodialysis Patients in Khuzestan Province, Southwest Iran *Saudi J Kidney Dis Transpl 2009;20(4):681-684*

76. Wreghitt TG. Blood-borne virus infections in dialysis units — A review. *Rev Med Virol 1999;9:101*

77. Ben Othman S, Bouzgarrou N, Achour A, Bourlet T, Pozzetto B,Trabelsi A. High prevalence and incidence of hepatitis C virus infections among dialysis patients in the East-Centre of Tunisia. *Pathol Biol (Paris) 2004;52:323-7.*

78.Schneeberger PM, Keur I, van Loon AM, Mortier D, de Coul KO,van Haperen AV, *et al.* The prevalence and incidence of hepatitisC virus infections among dialysis patients in the Netherlands: Anationwide prospective study. *J Infect Dis 2000;182:1291-9.*

79. Bassam Bernieh Viral hepatitis in hemodialysis: An update *Journal of translational internal medicine / Jul-Sep 2015 / vol 3 | issue **3***

80.Kalantar-Zadeh K, Miller LG, Daar ES. Diagnostic discordance forhepatitis C virus infection in hemodialysis patients. *Am J Kidney Dis2005;46:290-300.2005;46:290-300*

81.Dzekova-Vidimliski P., Asani A., Selim Gj., Gelev S., Polenakovic M., Sikole A. Patterns of viraemia in haemodialysis Patients with hepatitis c Contributions, *Sec. Biol. Med. Sci., MASA, XXIX, 2, p. 201–211 (2008)*

82. Saleem kamili ,Jan Drobenuic,Aufra C.Araujo,Tonya M Hayden , Lab diagnosis of Hepatitis C *Clinical infectious diseases 2012 ;55(s1)S43-8*

83. Fabrizi F, Lunghi G, Aucella F, Mangano S, Barbisoni F, Bisegna S, *et al*. Novel assay using total hepatitis C virus (HCV) core antigen quantification for diagnosis of HCV infection in dialysis patients. *J Clin Microbiol 2005;43:414-20*.

84. Recep Kesli, Hakkı Polat, Yuksel Terzi, Muhammet Guzel Kurtoglu, and Yavuz Uyar Comparison of a Newly Developed Automated and Quantitative Hepatitis C Virus (HCV) Core Antigen Test with the HCV RNA Assay for Clinical Usefulness in Confirming Anti-HCV Results *journal of clinical microbiology, dec. 2011, p. 4089–4093*

85. Courouce AM, Le Marrec NL, Bouchardeau F, Razer A, Maniez M, Laperche S, *et al*. Efficiency of HCV core antigen detection during the pre seroconversion period. *Transfusion* 2000;**40**:1198

86. Bukh J, Wantzin P, Krogsgaard K, Knudsen F, Purcell RH, Miller RH. High prevalence of hepatitis C virus (HCV) RNA in dialysis patients: failure of commercially available antibody tests to identifya significant number of patients with HCV infection. Copenhagen Dialysis HCV Study Group. *J Infect Dis* 1993; 168: 1343-1348

87. Al-Freah MA, Zeino Z, Heneghan MA. Management of hepatitis C in patients with chronic kidney disease. *Curr Gastroenterol Rep* 2012;**14**: 78-86.

88. . Almroth G, Ekermo B, Mansson AS, Svensson G, Widell A (2002) Detection and prevention of hepatitis C in dialysis patients and renal transplant recipients.A long-term follow up (1989–January 1997). *J Intern Med 251:119–128*

89. Seyed Moayed Alavian, Kamran bagheri-lankarani, Mitra Mahdavi-mazdeh, Shahram *Nourozi* . Hepatitis B and C in dialysis units in Iran:Changing the epidemiology *Hemodialysis International 2008; 12:378–382*

90.Salma *BdourJ* Hepatitis C virus infection in Jordanian haemodialysis units: serological diagnosis and genotyping *J. Med. Microbiol. — Vol. 51 (2002), 700–704*

91. Tajeldin M. Abdallah, Mubarak . Idriss, Alzubair M. Ahmed, Abdel- Aziem A. Ali and Osman K. Saeed Sero-Prevalence of Hepatitis B and Hepatitis C Viruses among Tuberculosis Patients in Kassala, Eastern Sudan *Global Journal of Infectious Diseases and Clinical Research*

92. Batool A, Khan MI, Bano KA. Efficacy of immunoassaychromatography test for hepatitis-C antibodies detection. *J Ayub Med Coll Abbottabad* 2009; 21: 38-39

93.Hayder I, Ahmed W, Alam SE. Comparison of Different ICT Kits for HBsAg and Anti HCV Using Gold Standard ELISA. *Pak J Med Res* 2012; 51: 72-76

94.Byce David Smith CDC initiative to prevent HCV infection update *Clinical infectious diseases July 2012*

95 . Liu CH, Kao JH. Treatment of hepatitis C virus infection in patients with end-stage renal disease. *J Gastroenterol Hepatol* 2011; **26**:228-239

96. Yen T, Keeffe EB, Ahmed A. The epidemiology of hepatitis C virus with infection. *J Clin Gastroenterol 2003;36:47-53*

97. Strader DB, Wright T, Thomas DL, Seeff LB. American Association for the Study of Liver Diseases: Diagnosis, management, and treatment of hepatitis C. *Hepatology 2004;39:1147-71.*

98 . Busch MP, Wilber JC, Johnson PJ, Tobler C, Evans CS: Impact of specimen handling and storage on detectionof hepatitis C virus RNA. *Transfusion 32:420-425, 1992*

99. Knipe ,David M,Howley ,Peter M Field's virology Hepatitis C virus Stanley M.Lemon,Christopher Walker,Miriam J .Alter,MinKyung Yi ,Fifth edition

100.Zein N,Clinical significance of hepatitis C virus genotypes ,*Clin Microbiology Rev,2000 Apr :13(2):223-35*

101. Sukanya Raghuraman a, R.V. Shaji c, Gopalan Sridharan a,Sujatha Radhakrishnan a, George Chandy b, B.S. Ramakrishna b, Priya AbrahamDistribution of the different genotypes of HCV among patients attending a tertiary care hospital in south *India Journal of Clinical Virology 26 (2003) 61–69*

102. Perez RM, Ferraz ML, Figueiredo MS, Contado D, Koide S,Ferreira AP, Cendoroglo Neto M, Medina Pestana JO, Silva AE. Unexpected distribution of hepatitis C virus genotypes in patients on hemodialysis and kidney transplant recipients. *J Med Virol* 2003; 69: 489-494

103. Kamyar Kalantar-Zadeh Æ Eric S. Daar Æ Viktor E. Eysselein Æ Loren G. Miller Hepatitis C inflection in dialysis patients: a link to poor clinical outcome?

Int Urol Nephrol (2007) 39:247–259

*104.*Norberto Perico,Dario Cattan, Boris Bikbov,and Giuseppe Remuzzi Hepatitis C Infection and Chronic Renal Diseases *Clin J Am Soc Nephrol* 4: 207–220, 2009

105.Goodman and Gilman's The Pharmacological basis of Therapeutics Laurence L.Brunton John S.Lazo ,Keith L. Parker Eleventh edition

106.Khasia Anwar,Muhammad Imran,Farhana Shahzad,Mamoona Noreen,Muhammad Atif,Fayyaz Ahmed et al Prevalence of Hepatitis B and Hepatitis C Infection among Patients Undergoing Dialysis *Journal of Human Virology & Retrovirology Volume 3 Issue 3 - June 06*, 2016.

107. Shabana Razmin, M.Reenaa ,Vishnu Prasad, P.Rajendran Hepatitis c and b virus infection among chronic renal failure patients undergoing haemodialysis in calicut, kerala state ***Asia Pacific Journal of Research Vol: I Issue XI, November 2013***

108.Chan TM, Lok ASF and Cheng IKP. Hepatitis C infection among dialysis patients: A comparison between patients on maintenance haemodialysis and continuous ambulatory peritoneal dialysis. *Nephrol Dial Transplant 1991; 6: 944-947.*

109.Besso L, Rovere A, Pearno G, et al. Prevalence of HCV antibodies in a uraemic population undergoing maintenance dialysis therapy and in staff members of the dialysis unit. *Nephron 1992; 61: 304-306.*

110.Conway M, Catherall AP, Brown EA et al. Prevalence of antibodies to hepatitis C in dialysis patients and transplant recipients with possible routes of transmission. *Nephrol Dial Transplant 1992*; *7: 1226-1229.*

111. Wang SM, Liu JH, Chou CY, Huang CC, Shih CM, Chen W.Mortality in hepatitis C-positive patients treated with peritoneal dialysis. *Perit Dial Int 2008; 28:183–7.*

112.Huang CC, Wu MS, Lin DY, Liaw YF. The prevalence of hepatitisC virus antibodies in patients treated with continuous ambulatory peritoneal dialysis. *Perit Dial Int 1992; 12:31–3.*

113.Jia-Jung Lee, Ming-Yen Lin, Jung-San Chang, Chi-Chih Hung, Jer-Ming Chang,Hung-Chun Chen, Ming-Lung *et al* Hepatitis C Virus Infection Increases Risk of Developing End-Stage Renal Disease Using Competing Risk Analysis *PLOS ONEJune 2014 | Volume 9 | Issue 6 | e1007904*.

114.Abdulkarim AS, Zein NN, Germer JJ et al. Hepatitis C virus genotypes and hepatitis G virus in hemodialysis patients fromSyria: identification of two novel hepatitis C virus subtypes.*Am J Trop Med Hyg 1998; 59: 571–5767*.

115. .Rigopoulou EI, Stefanidis I, Liaskos C, Zervou EK, Rizos C,Mina P, *et al.* HCV-RNA qualitative assay based on transcription mediated amplification improves the detection of hepatitis C virus infection in patients on hemodialysis: results from five hemodialysis units in central Greece. *J Clin Virol* 2005; **34**: 81-5.

116 . **Hanuka, N., E. Sikuler, D. Tovbin, M. Mostoslavsky, M. Hausman, M. Ogel,A. Yaari, and Y. Shemer-Avni.** 2002. Hepatitis C virus infection in renalfailure patients in the absence of anti-hepatitis C virus antibodies. J. *Viral Hepat.* ***9:****141–145.*

117.Pawlotsky, J.M. 2002. Use and interpretation of virological tests for hepatitis C. *Hepatology,* ***36****(5): 65–73.*

118.Al Meshari, K., Al Ahdal, M., Alfurayh, O., Ali, A., De Vol, E.,and Kessie, G. 1995. New insights into Hepatitis C virus infection of hemodialysis patients: the implications. *Am. J. Kidney Dis.* ***25****: 572–578.*

119.Garinis G, Spanakis N, Theodorou V, Gorgoulis V, Manolis E,Karameris A, *et al.* Comparison of the enzyme-linked immunosorbant assay III, recombinant immunoblot third generation assay, and polymerase chain reaction method in the detection of hepatitis C virus infection in haemodialysis patients. *J Clin Lab Anal 1999;13:122-5*

120.Sullivan DG, Kim SS, Wilson JJ, Stehman-Breen C, Gretch DR.
Investigating hepatitis C virus heterogeneity in a high prevalence setting
using heteroduplex tracking analysis. *J Virol Methods 2001;96:5-16.*.

121.Bouzgarrou N, Fodha I, Othman SB, Achour A, Grattard F,Trabelsi A, *et al.* Evaluation of a total core antigen assay for the diagnosis of hepatitis C virus infection in hemodialysis patients. *J Med Virol* 2005; **77**: 502-8.

122.Salama G, Rostaing L, Sandres K, Izopet J:Hepatitis C virus infection in French hemodialysis units: a multicenter study. *J Med Virol 2000; 61: 44–51*

123.Mendez-Sanchez N, Motola-Kuba D, Chavez- Tapia NC, Bahena J, Correa-Rotter R, Uribe M: Prevalence of hepatitis C virus infection among hemodialysis patients at a tertiarycare hospital in Mexico City, Mexico. *J Clin Microbiol 2004; 42: 4321–4322*

124. Talia Weinstein , Ran Tur-Kaspa , Avry Chagnac , Asher Korzets , Yacov Ori Dina Zevin *et al* Hepatitis C Infection in Dialysis Patients in Israel *IMAJ 2001;3:174-177*

125. Moreira RC, Lemos MF, Longui CA, Granato C: Hepatitis C and Hemodialysis: A Review. *The Brazilian Journal of Infectious Diseases 2005,*

9(3):269-27

126.Ijaz Ali, Lubna Siddique, Latif U Rehman, Najib U Khan, Aqib Iqbal, Iqbal Munir *et al* Prevalence of HCV among the high risk groups in Khyber Pakhtunkhwa *Virology Journal 2011, 8:29*

127.Khan J.K., Lone D.S., Hameed A.,Munim M.R., Bhatti M.,Khattak A.A. *et al* Evaluation of the Performance of Two Rapid Immunochromatographic Tests for Detection of Hepatitis B Surface Antigen and Anti HCV Antibodies Using Elisa Tested *Samples Special Edition Annals Vol 16. No.1 Jan. - Mar. 2010*

128.Ijaz Hayder, Waquaruddin Ahmed, Syed Ejaz AlamComparison of Different ICT Kits for HBsAg and Anti HCV Using Gold Standard ELISA *Pak J Med Res Vol. 51, No. 3, 2012*

129.N. Muhammad, frequency of hepatitis c in buner, nwfp. *J coll physicians surg pak. 15, 11–4 (2005)*

130. Kranthi Kosaraju,Sameer Singh Faujdar, Aashima Singh, and Ravindra Prabhu Hepatitis Viruses in Heamodialysis Patients:An Added Insult to Injury? *Hindawi Publishing Corporation Hepatitis Research and Treatment*
Volume 2013, Article ID 860514, 4 pages

131.Saxena AK, Panhotra BR. The impact of nurse understaffing on the transmission of hepatitis C virus in a hospital-based hemodialysis unit. *Med Princ Pract. 2004;13:129–135*

132. Ayman karkar Hepatitis C in dialysis units: The Saudi experience *Hemodialysis International 2007; 11:354–3610.*

133.Ibrahim Ummate, Ballah Akawu Denue,&, Ibrahim Musa Kida, Onah Joseph Ohioma, David Bukbuk, Baba, Waru Goni

Risk factors for hepatitis C virus sero-positivity among haemodialysis patients receiving care at Kidney Centre in a tertiary health facility in Maiduguri, Nigeria

Pan African Medical Journal. 2014; 19:305

134. Bhawmik P,Debnath K,Prevalence of Hepatitis C and Hepatitis B among hemodialysis patients of Tripura,*India Euroasian J Hepato-Gastroentrology,2012 2(1),10-13*

135. Prasad KP Babu, Parameswari Katay, Geethanjali Anke Seroprevalence of HIV, HBV & HCV in High Risk Groups

Indian Journal of Medical Research and Pharmaceutical Sciences August 2015; 2(8)

136.Dussol B, Berthezene P, Brunet P *et al.* Hepatitis C virus Infection among chronic dialysis patients. In the south ofFrance: A Collaborative Study. *American Journal of Kidney Diseases 1995; 25(3): 399-404.*

137.Zacks SL and Fried MW. Hepatitis B and C and renalfailure. *Infect Dis Clin North Am 2001; 15(3): 877-99.*

138.Al-Jiffri AMY, Fadeg RB, Ghabrah TM, Ibrahim A. Hepatitis C virus infection among patients on hemodialysis in Jeddah: A single center experience. *Saudi J Kidney DisTransplant. 2003; 14:84–89*

139. Al-Jamal M, Al-Qudah A, Al-Shishi KF, Al-Sarayreh A, Al-Quraan L: Hepatitis C virus (HCV) infection in hemodialysis patients in the south of Jordan.

Saudi J Kidney Dis Transpl 2009, 20:488-92.

140. Sypsa V, Psichogiou M, Katsoulidou A, SkoutelisG, Moutafis S, Hadjiconstantinou V *et al.* Incidenceand patterns of hepatitis C virus seroconversion in a cohort of hemodialysis patients. *Am J Kidney Dis2005;45:334-43*

141. Wiam A Alashek, Christopher W McIntyre, Maarten W Taal Hepatitis B and C infection in haemodialysispatients in Libya: prevalence, incidence and risk *factorsBMC Infectious Diseases 2012, 12:265*

142.Nakai S, Masakane I, Akiba T, Iseki K, Watanabe Y, Itami N, et al. Overview of regular dialysis treatment in Japan (as of 31December 2005). *Ther Apher Dial. 2007;11:411–41.*

143. Hossein Khedmat1, Mohsen Amini1, Mohammad Ebrahim Ghamar-Chehreh1, Shahram Agah2**Hepatitis C Virus Infection in Dialysis Patients** *Saudi J Kidney Dis Transpl 2014;25(1):1-8*

144.Waqar M, Khan AU, Rehman HU, Idrees M, Wasim M, AliA, Niaz Z *et al* Determination of hepatitis C virus genotypes circulating in different districts of Punjab (Pakistan). *Eur J Gastroenterol Hepatol* 2014; **26**: 59-64

145.**Ali S**, Ahmad A, Khan RS, Khan S, Hamayun M, Khan SA *et al*, Genotyping of HCV RNA reveals that 3a is the most prevalent genotype in mardan, pakistan. *Adv Virol* 2014

146. Muhammad Sohail Afzal, Muhammad Yousaf Khan, Muhammad Ammar, Sadia Anjum, Najm us Sahar Sadaf Zaidi Diagnostically untypable hepatitis C virus variants: It is time to resolve the problem*World J Gastroenterol 2014 Dec 14; 20(46): 17690-17692*

147. Hairul Aini H, Mustafa M I , Seman M R, Nasuruddin B A,Mixed-genotypes Infections with Hepatitis C Virus inHemodialysis Subjects *Med J Malaysia Vol 67 No 2 April 2012*

148. Sadia Butt, Muhammad Idrees, Irshad Ur Rehman, Haji Akbar, Muhammad Shahid, Samia Afzal, *et al* Mixed genotype infections with HCV,Pakistan *Emerg Infect Dis. 2011 Aug; 17(8): 1565–1567.*

149.Qian KP, Natov SN, Pereira BJ, Lau JY. Hepatitis C virus mixed genotype infection in patients on haemodialysis. *J Viral Hepat 2000; 7(2): 153-60.*

PROFORMA

Name :

Age :

Sex :

Occupation :

Address :

Contact no. :

Height : Weight :

Diagnosis :

Basic disease leading to renal failure :

Co-morbid conditions :

H/o blood transfusions

When	How many times	Screened/Unscreened

H/o Surgery :

H/o blood contact :

Accidental	Dental procedures	Blood,mucous exposure

H/o Jaundice/Cirrhosis :

H/o injections im/iv :

H/o STD :

H/o IV drug abuse :

H/o Acupuncture/Tattooing :

H/o bleeding disorders /Anemia :

H/o vaccination :

H/o parents died of jaundice :

H/o Organ transplant :

H/o spouse HCV/household contact :

Type of Dialysis :

H/o Previous Hemodialysis :

1.First Hemodialysis :

2.No. of Hemodialysis :

3.Duration of Hemodialysis :

4.Frequency of Hemodialysis :

Once weekly

Twice weekly

Thrice weekly

5.Nature of hemodialysis :

6.AV Fistula made :

7.How many times :

8. No of Dialysis Centres Visited :

9.Dialyser :Re -use Non Re-use

General examination :

Lab investigations :

Hb : LFT:

RBS: Blood grouping:

RFT :

HCV Card test: Anti –HCV Elisa:

RT- PCR :

Genotype :

Colour plae:1

HCV rapid ICT kit

Colour Plate: 2

Card test positive for anti-HCV

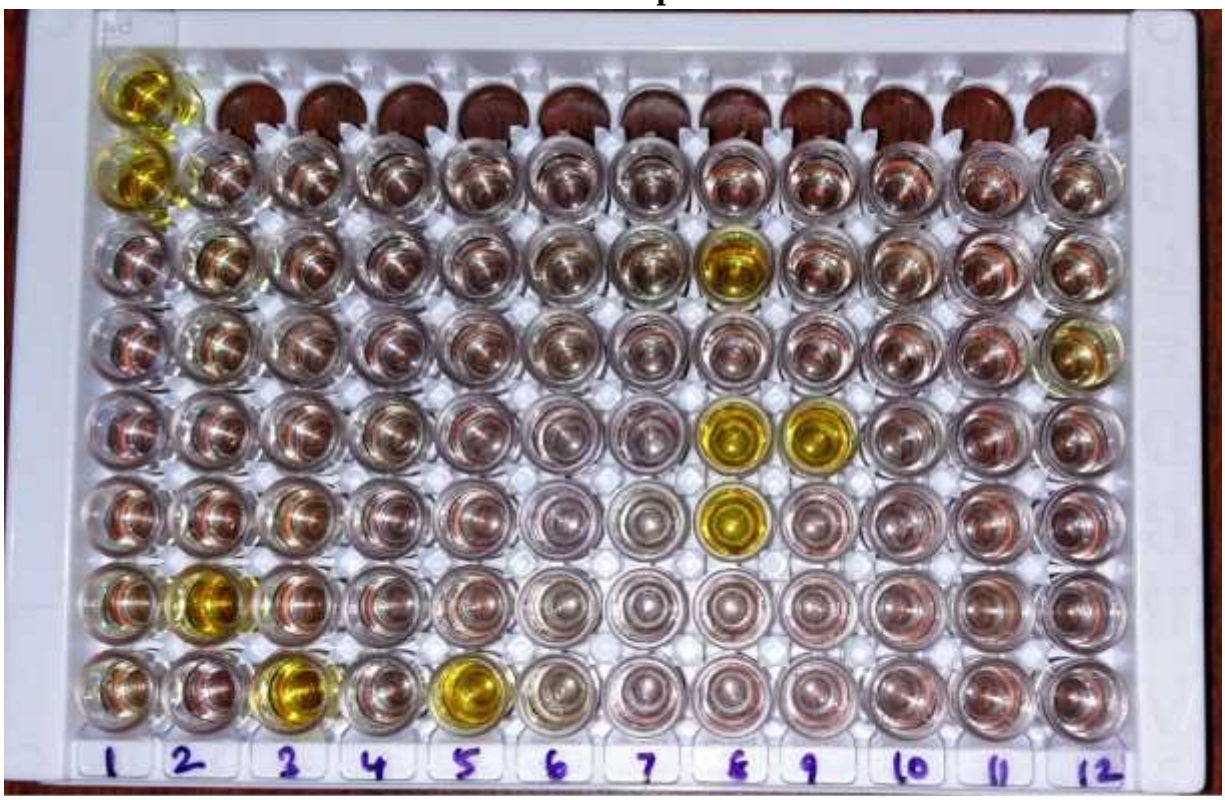
Card test negative for anti-HCV

Positive Negative

Colour Plate:3

Third generation anti-HCV ELISA kit

Micro-titre plate



Colour Plate:4

ELISA Reader

Colour Plate:5

RNA Extraction kit

HCV Genotyping Real-time PCR kit

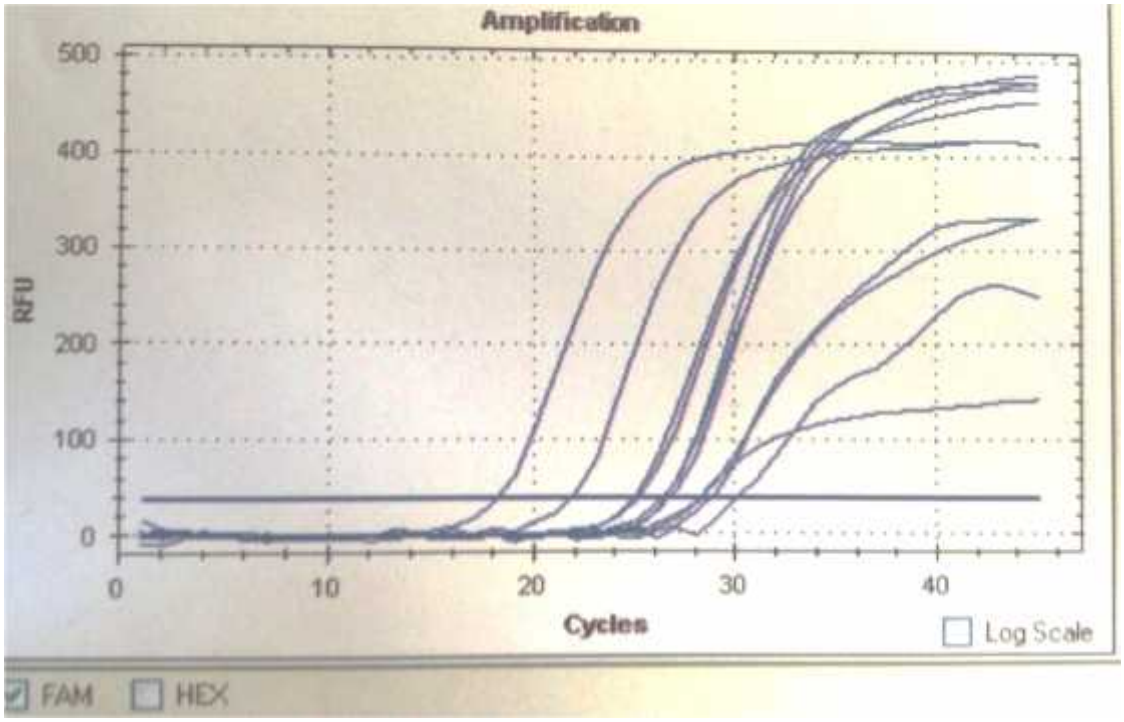
COLOUR PLATE 6

Molecular Detection by PCR

Thermocycler

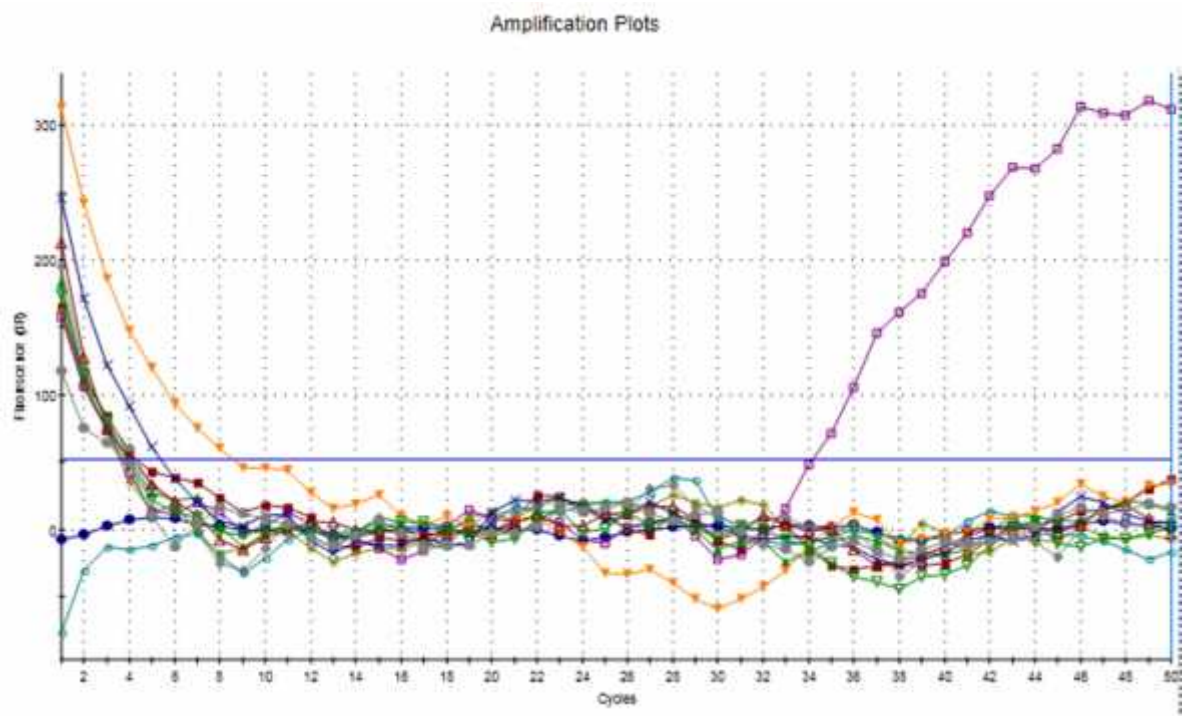
Colour Plate: 7

Amplification curve showed amplification for HCV RNA

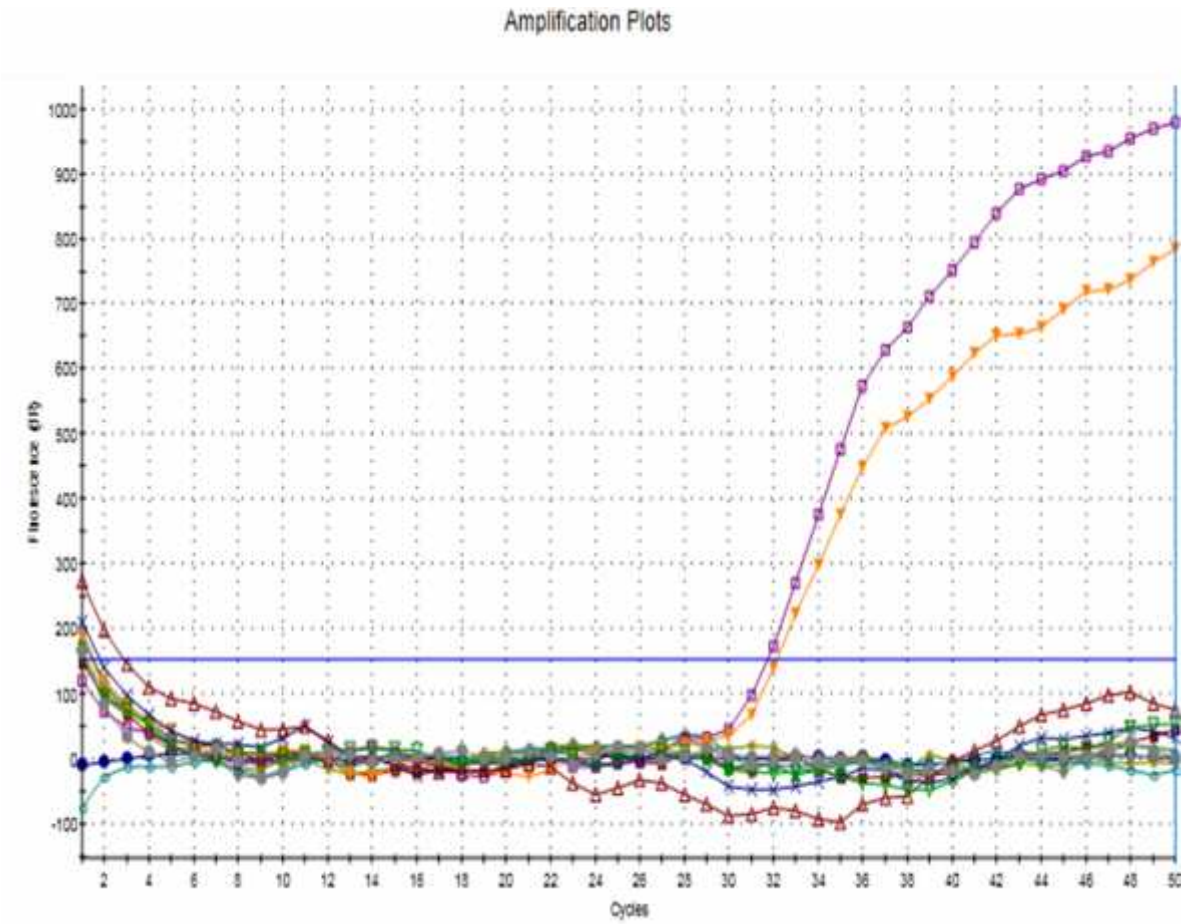


Colour Plate:8

Amplification curve showed genotype 1

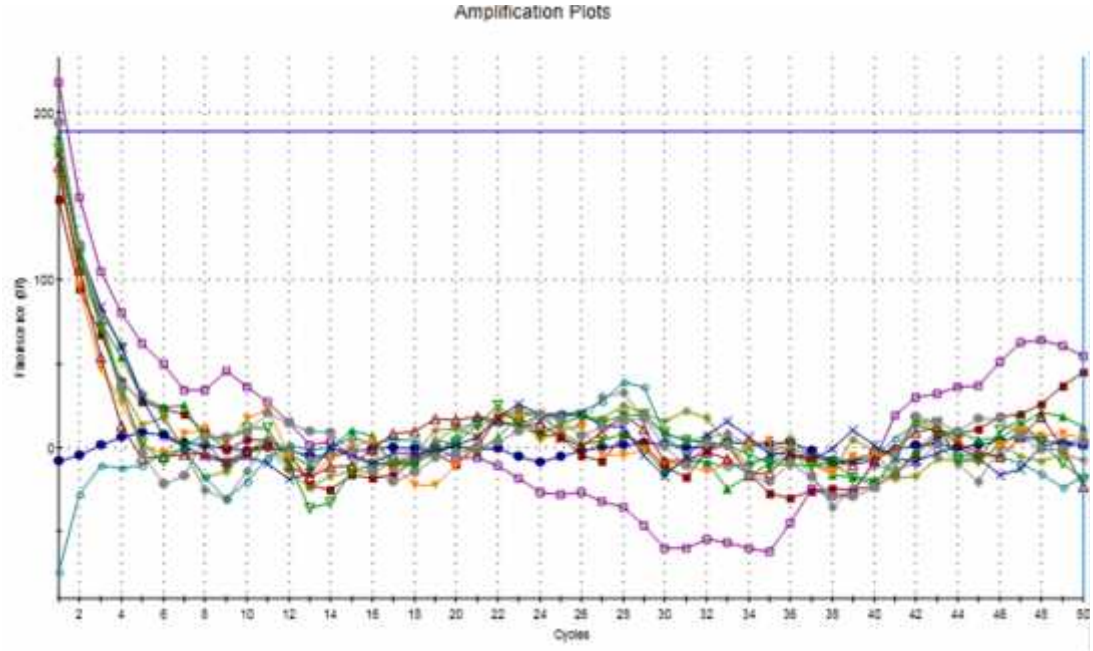


Amplification curve showed Mixed genotype 1,2



Colour Plate:9

Amplification curve showed Untypable genotype



S.No	AGE	SEX	DIAGNOSIS	ETIOLOGY	MODE	DURATION	FREQUENCY	NUMBER	No. OF CENTRES	AVF/ TIMES	DIALYSER REUSE	BLOOD TRANSFUSION	BLOOD CONTA	IM/IV	ORGAN TRANSPLANT	JAUNDICE	OTHERS	ALT	ICT	ELISA	PCR	GENO
1	28	FEMALE	CKD	CGN	PD,HD	21 MON	TWICE	31	2	1	R	Y<3	N	N	N	N	N	LOW NL	NEG	NEG	NEG	NA
2	47	FEMALE	CKD	DM	PD	6 MON	NA	5 CYCLE	1	0	0	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
3	47	FEMALE	CKD	DM	HD	7 MON	TWICE	38	1	1	R	N	N	N	N	N	Y(TATTOOING)	HIGH NL	NEG	NEG	NEG	NA
4	52	FEMALE	CKD	HTN	PD,HD	21 MON	TWICE	76	2	2	R	Y<3	N	N	N	N	Y(TATTOOING)	ELEVATED	NEG	NEG	NEG	NA
5	34	MALE	CKD	HTN	HD	7 MON	ONCE	54	1	2	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
6	57	MALE	CKD	DM	HD	9 MON	TWICE	62	2	1	R	N	N	N	N	Y	Y(TATTOOING)	HIGH NL	NEG	NEG	NEG	NA
7	48	FEMALE	CKD	DM	PD,HD	20 MON	TWICE	81	2	2	R	Y<3	N	N	N	N	Y(SURGERY)	LOW NL	NEG	NEG	NEG	NA
8	41	MALE	CKD	HTN	HD	7 MON	ONCE	54	1	2	R	Y<3	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
9	24	FEMALE	CKD	CGN	PD,HD	24 MON	TWICE	120	2	3	R	y3-6	N	N	N	Y	n	LOW NL	POS	POS	NEG	NA
10	42	MALE	CKD	HTN	HD	20 MON	TWICE	97	1	1	R	N	N	N	N	N	Y(SURGERY)	HIGH NL	NEG	NEG	NEG	NA
11	45	MALE	CKD	DM	HD	6 MON	ONCE	38	1	1	R	Y<3	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
12	36	MALE	CKD	HTN	HD	19 MON	TWICE	104	2	2	R	Y<3	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
13	29	FEMALE	CKD	HTN	PD,HD	40 MON	TWICE	84	1	2	R	Y<3	N	N	N	N	N	LOW NL	NEG	NEG	NEG	NA
14	15	MALE	CKD	OTHERS	PD,HD	12 MON	TWICE	78	1	2	R	Y<3	N	N	N	Y	N	HIGH NL	NEG	NEG	NEG	NA
15	42	MALE	CKD	HTN	HD	21 MON	TWICE	102	1	1	R	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
16	60	FEMALE	CKD	HTN	PD,HD	6 MON	ONCE	45	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
17	35	MALE	CKD	HTN	HD	22 MON	TWICE	135	2	1	R	N	N	N	N	N	N	HIGH NL	POS	POS	POS	Untypable
18	39	FEMALE	CKD	HTN	HD	22 MON	TWICE	126	1	2	R	y<3	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
19	33	MALE	CKD	HTN	PD,HD	16 MON	ONCE	60	1	2	R	N	Y(DENTAL	N	N	N	Y(BARBER)	LOW NL	NEG	NEG	NEG	NA
20	41	FEMALE	CKD	DM	PD,HD	42 MON	ONCE	66	2	2	R	N	N	N	N	N	Y(TATTOOING)	HIGH NL	NEG	NEG	NEG	NA
21	58	MALE	CKD	DM	HD	9 MON	TWICE	52	1	1	R	Y<3	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
22	37	MALE	CKD	HTN	PD	6 MON	NA	5 CYCLES	1	0	0	Y<3	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
23	23	MALE	CKD	CGN	PD,HD	20 MON	ONCE	122	1	2	R	N	N	N	N	N	Y(TATTOOING)	HIGH NL	NEG	NEG	NEG	NA
24	38	FEMALE	CKD	HTN	HD	9 MON	ONCE	36	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
25	62	MALE	CKD	DM	PD,HD	18 MON	ONCE	90	2	2	R	Y<3	N	N	N	N	Y(BARBER)	LOW NL	NEG	NEG	NEG	NA
26	40	MALE	CKD	HTN	HD	12 MON	TWICE	56	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
27	42	MALE	CKD	HTN	PD,HD	16 MON	ONCE	70	2	2	R	Y<3	N	N	N	N	Y(TATTOOING)	HIGH NL	NEG	NEG	NEG	NA
28	33	FEMALE	CKD	HTN	PD,HD	8 MON	ONCE	34	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
29	17	MALE	CKD	CGN	PD,HD	9 MON	ONCE	67	1	1	R	y<3	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
30	45	MALE	CKD	HTN	HD	8 MON	TWICE	65	1	1	R	N	N	N	N	N	Y(BARBER)	ELEVATED	NEG	NEG	NEG	NA
31	48	MALE	CKD	HTN	HD	21 MON	TWICE	115	1	3	R	N	N	N	N	N	Y(BARBER)	LOW NL	POS	POS	POS	1
32	55	FEMALE	CKD	DM	HD	7 MON	TWICE	30	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
33	23	FEMALE	CKD	CIN	PD,HD	8 MON	ONCE	44	2	1	R	y<3	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
34	40	MALE	CKD	HTN	PD	12 MON	NA	10 CYCLES	2	0	0	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
35	49	MALE	CKD	HTN	HD	8 MON	ONCE	40	1	1	R	N	Y(DENTAL	N	N	N	Y(BARBER)	LOW NL	NEG	NEG	NEG	NA
36	60	FEMALE	CKD	DM	HD	6 MON	ONCE	28	1	1	R	N	N	N	N	N	Y(TATTOOING)	HIGH NL	NEG	NEG	NEG	NA
37	48	MALE	CKD	DM	PD,HD	9 MON	TWICE	48	1	1	R	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
38	26	FEMALE	CKD	CGN	PD,HD	10 MON	TWICE	51	2	1	R	N	N	N	N	N	N	LOW NL	NEG	NEG	NEG	NA
39	52	FEMALE	CKD	DM	PD,HD	17 MON	TWICE	30	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
40	38	MALE	CKD	HTN	PD	8 MON	NA	7 CYCLES	1	0	0	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
41	36	MALE	CKD	CGN	PD,HD	21 MON	TWICE	132	1	2	R	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
42	52	FEMALE	CKD	HTN	PD,HD	22 MON	TWICE	156	3	2	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
43	31	MALE	CKD	HTN	HD	9 MON	TWICE	42	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
44	20	FEMALE	CKD	HTN	HD	8 MON	TWICE	44	1	1	R	N	N	N	N	Y	N	HIGH NL	NEG	NEG	NEG	NA
45	49	MALE	CKD	HTN	HD	18 MON	TWICE	80	2	2	R	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
46	27	MALE	CKD	HTN	HD	5 MON	TWICE	40	1	1	R	N	N	N	N	N	Y (BARBER)	HIGH NL	NEG	NEG	NEG	NA
47	24	MALE	CKD	CIN	HD	32 MON	TWICE	186	3	2	R	N	N	N	N	Y	N	HIGH NL	POS	POS	POS	1
48	33	FEMALE	CKD	HTN	HD	13 MON	ONCE	84	2	2	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
49	38	FEMALE	CKD	DM	PD,HD	26 MON	TWICE	184	1	2	R	Y3-6	N	N	N	N	N	LOW NL	POS	POS	POS	1
50	34	MALE	CKD	HTN	HD	30 MON	TWICE	176	1	2	R	N	Y(DENTAL	N	N	N	N	LOW NL	POS	POS	POS	1
51	48	MALE	CKD	CGN	HD	10 MON	TWICE	46	1	1	R	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
52	48	MALE	CKD	DM	HD	7 MON	TWICE	30	1	2	R	N	N	N	N	N	n	HIGH NL	NEG	NEG	NEG	NA
53	42	MALE	CKD	DM	PD,HD	10 MON	ONCE	58	2	2	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
54	43	MALE	CKD	HTN	PD	8 MON	NA	6 CYCLES	1	0	0	Y3-6	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
55	61	MALE	CKD	DM	PD	9 MON	NA	4 CYCLES	1	0	0	N	Y(DENTAL	N	N	N	N	LOW NL	NEG	NEG	NEG	NA
56	25	MALE	CKD	OTHERS	HD	20 MON	TWICE	104	1	2	R	Y<3	N	N	N	Y	Y(BARBER)	HIGH NL	POS	POS	NEG	NA
57	38	FEMALE	CKD	CGN	HD	17 MON	ONCE	43	2	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
58	37	MALE	CKD	HTN	HD	16 MON	ONCE	52	1	1	R	Y3-6	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
59	42	MALE	CKD	HTN	HD	7 MON	ONCE	26	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
60	31	MALE	CKD	OTHERS	HD	8 MON	TWICE	45	3	1	R	N	N	N	N	N	N	ELEVATED	NEG	NEG	POS	type 1
61	57	MALE	CKD	DM	PD	7 MON	NA	4 CYCLES	1	0	0	N	N	N	N	N	Y(BARBER)	LOW NL	NEG	NEG	NEG	NA
62	36	MALE	CKD	HTN	HD	17 MON	ONCE	60	2	1	R	N	N	N	N	Y	N	ELEVATED	NEG	NEG	NEG	NA
63	72	MALE	CKD	DM	HD	6 MON	TWICE	43	1	1	0	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
64	46	FEMALE	CKD	CGN	PD	8 MON	NA	5 CYCLES	1	0	0	N	N	N	N	N	N	LOW NL	NEG	NEG	NEG	NA
65	48	MALE	CKD	CIN	PD	7 MON	NA	3 CYCLES	1	0	0	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
66	26	FEMALE	CKD	CGN	HD	7 MON	TWICE	58	1	2	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
67	33	FEMALE	CKD	DM	PD	5 MON	NA	3 CYCLES	1	0	0	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
68	25	MALE	CKD	OTHERS	HD	5 MON	ONCE	44	1	1	R	N	N	N	N	N	Y(BARBER)	LOW NL	NEG	NEG	NEG	NA
69	44	FEMALE	CKD	HTN	HD	6 MON	TWICE	78	2	1	R	Y<3	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
70	14	FEMALE	CKD	CGN	PD,HD	24 MON	ONCE	40	3	1	R	y<3	N	N	N	N	Y(SYRGERY)	HIGH NL	NEG	NEG	NEG	NA

71	38	MALE	CKD	HTN	HD	24 MON	TWICE	140	1	2	R	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
72	30	FEMALE	CKD	CIN	PD	6 MON	NA	4 CYCLES	1	0	0	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
73	35	MALE	CKD	HTN	HD	15 MON	ONCE	100	4	3	R	y>6	N	N	Y	N	Y(SURGERY)	LOW NL	NEG	NEG	NEG	NA
74	57	FEMALE	CKD	DM	HD	12 MON	TWICE	76	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
75	58	MALE	CKD	HTN	PD,HD	8 MON	ONCE	54	1	1	R	N	Y(DENTAL	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
76	38	MALE	CKD	HTN	HD	8 MON	TWICE	66	1	1	R	N	N	N	N	N	N	LOW NL	NEG	NEG	NEG	NA
77	37	MALE	CKD	HTN	HD	9 MON	ONCE	42	1	1	R	N	Y(DENTAL	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
78	48	MALE	CKD	HTN	PD,HD	28 MON	ONCE	96	2	2	R	N	N	N	N	N	N	LOW NL	NEG	NEG	NEG	NA
79	40	MALE	CKD	HTN	HD	8 MON	TWICE	65	1	2	R	N	N	N	N	Y	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
80	44	MALE	CKD	HTN	PD,HD	7 MON	TWICE	40	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
81	19	FEMALE	CKD	CGN	PD,HD	28 MON	THRICE	60	4	3	R	y>6	N	N	Y	N	Y(SURGERY)	HIGH NL	NEG	POS	POS	mixed 1,2
82	26	MALE	CKD	HTN	HD	8 MON	TWICE	32	1	1	R	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
83	35	MALE	CKD	HTN	PD	6 MON	NA	5 CYCLE	1	0	0	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
84	43	MALE	CKD	DM	HD	8 MON	ONCE	42	1	1	R	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
85	23	MALE	CKD	HTN	HD	12 MON	TWICE	75	1	2	R	y<3	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
86	22	MALE	CKD	CGN	HD	14 MON	THRICE	130	2	2	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
87	50	MALE	CKD	HTN	HD	7 MON	TWICE	32	1	1	R	N	N	N	N	N	Y(BARBER)	ELEVATED	NEG	NEG	NEG	NA
88	38	FEMALE	CKD	DM	PD	9 MON	NA	5 CYCLES	1	0	0	N	N	N	N	N	N	ELEVATED	NEG	NEG	NEG	NA
89	63	MALE	CKD	HTN	HD	6 MON	ONCE	38	1	2	R	y<3	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
90	41	FEMALE	CKD	HTN	HD	8 MON	TWICE	66	1	1	R	y<3	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
91	39	FEMALE	CKD	HTN	HD	7 MON	TWICE	46	1	1	R	N	N	N	N	Y	Y(TATTOOING)	HIGH NL	NEG	NEG	NEG	NA
92	38	MALE	CKD	HTN	HD	14 MON	TWICE	60	1	2	R	N	N	N	N	N	Y(BARBER)	LOW NL	NEG	NEG	NEG	NA
93	40	MALE	CKD	DM	HD	5 MON	ONCE	34	1	2	R	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA
94	48	FEMALE	CKD	DM	PD,HD	8 MON	TWICE	44	2	1	R	y<3	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
95	27	MALE	CKD	OTHERS	HD	10 MON	TWICE	54	1	2	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
96	19	MALE	CKD	OTHERS	HD	6 MON	ONCE	44	1	1	R	N	N	N	N	N	N	ELEVATED	NEG	NEG	NEG	NA
97	34	MALE	CKD	OTHERS	HD	5 MON	TWICE	32	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
98	64	FEMALE	CKD	HTN	HD	7 MON	ONCE	28	2	1	R	N	N	N	N	N	Y(TATTOOING)	LOW NL	NEG	NEG	NEG	NA
99	35	MALE	CKD	CIN	PD,HD	9 MON	TWICE	27	1	1	R	N	N	N	N	N	N	HIGH NL	NEG	NEG	NEG	NA
100	32	MALE	CKD	HTN	PD,HD	11 MON	ONCE	33	1	1	R	N	N	N	N	N	Y(BARBER)	HIGH NL	NEG	NEG	NEG	NA